Potential for Treatment of Agricultural Drain Water with Microalgal-Bacterial Systems

August 1985

U. S. Department of the Interior
Bureau of Reclamation

William J. Oswald

This report presents the results of a study undertaken to determine the potential for use of microalgal-bacterial systems to treat agricultural drainage water. The study was funded by the U.S. Bureau of Reclamation as part of the Federal-State Interagency San Joaquin Valley Drainage Program. Publication of the findings and recommendations herein should not be construed as representing the concurrence of either the Bureau of Reclamation or any other Federal or State agency participating in the Drainage Program. Also, mention of trade names or commercial products does not constitute endorsement or recommendation by the agencies. The purpose of this report is to provide the Drainage Program agencies with information and alternatives for further consideration.

The San Joaquin Valley Drainage Program was established in mid-1984 and is a cooperative effort of the U.S. Bureau of Reclamation, U.S. Fish and Wildlife Service, U.S. Geological Survey, California Department of Fish and Game, and California Department of Water Resources. The purposes of the Program are to investigate the problems associated with the drainage of agricultural lands in the San Joaquin Valley and to develop solutions to those problems. Consistent with these purposes, Program objectives address the following key areas: (1) Public health, (2) surface- and ground-water resources, (3) agricultural productivity, and (4) wildlife resources.

Inquiries concerning the San Joaquin Valley Drainage Program may be directed to:

San Joaquin Valley Drainage Program Federal-State Interagency Study Team 2800 Cottage Way, Room W-2143 Sacramento, California 95825-1898

Potential for Treatment of Agricultural Drain Water with Microalgal-Bacterial Systems

Prepared for

U.S. Department of the Interior Bureau of Reclamation Mid-Pacific Region 2800 Cottage Way Sacramento, California 95825

Ву

William J. Oswald, PhD, P.E. 1081 St. Francis Drive Concord, California 94518

Contract No. 5-PG-20-06820

August 1985



TABLE OF CONTENTS

			Page No.
Executive Summary			vii
Introduction			1
Review of Literature			2
Characteristics of Advanced High-Rate Ponds			11
Algae Separation			28
The High-Rate Pond in Selenium Removal			33
The Pilot System			47
High-Rate Pond Design			53
Facultative Pond Design			56
Dissolved Air Flotation Design			57
Operations			60
Study Plan for Pilot System		٠	62
Cost Estimate for 1-MGD System			64
Value of Energy			67
Discussion			67
Conclusions			68
Acknowledgement			69

Appendix A, Statement of Work Appendix B, Pourbaix Diagram Appendix C, References

FIGURES

No.	Title	Page No.
1	Simplified Diagram of Endless Channel Used for Algal Cultivation, Showing Nomenclature of Major Parts	13
2	Manufactured Fiberglass Paddle Wheel for Algae Growth Pond	15
3	Idealized and Linearized Model of the Influence of Solar Energy on Algal Productivity in Continuously Mixed, Light-limited Shallow Pond	25
4	Algal Productivity in Various Geographical Locations	27
5	Total Chromium Removal in Series Ponds	38
6	Schematic Diagram of Candidate Integrated Ponding System for Heavy Metal and Selenium Removal from Subsurface Tile Drainage Water	50
7	Cross Section of High-rate Ponds, Showing Levee and Berm Detail	54
8	Layout for One or Two Pairs of High-rate Ponds	55
9	Profiles of Facultative and Digestion Ponds 9-A and 9-B	58
10	Dissolved Air Flotation Unit for Algal Concentration	59

TABLES

No.	Title	Page No
I	Estimated Mean Value for Manning's "N" in Shallow Open Channels	17
II	Productivity of Microalgae Growing on Domestic Sewage in High-rate Ponds	26
III	Concentrations of Selenium Reported in Microalgae Grown in Central Valley Drainage Waters	34
IV	Sensitivity of Selected Microalgae to Selenium, Arsenic, Cadmium and Mercury	41
V	Comparison of Trace Constituents in Drainage Waters in Kesterson Reservoir, the DWR Desalting Facility at Los Banos and the Firebaugh Test Site, with Water Quality Standards for Fresh Water Aquatic Life, Drinking Water and Irrigation Water	44
VI	Range of Individual Constituents Reported in San Joaquin Valley Agricultural Drainage Waters	46
VII	Algae Biomass Potential of Unfertilized Drainage Water Showing Fertilizers to be Added to Make a Balanced Water for 300 Milligrams per Liter of Microalgae Dry Weight Basis	48



EXECUTIVE SUMMARY

It is not possible to determine from the available literature the extent of selenium removal that can be achieved in well-designed high-rate algal growth ponds alone. The amount can only be determined by in situ experiments with indigenous algae.

High-rate algal growth ponds are likely to contribute importantly in the treatment for other selenium removal systems by softening, clarifying and removing nutrients from drainage waters.

If used as treatment systems for drainage waters, high-rate algal ponds, in conjunction with in-pond methane fermentation, gas capture, and heat power generation will easily produce their own electrical energy needs and significant amounts of surplus electrical energy.

High-rate algal growth ponds, in conjunction with algal fermentation and acid gas scrubber systems, have a high probability of removing significant amounts of selenium from drainage waters. The amount can only be determined by in situ experiments with drainage water.

The cost of removing selenium, other toxic metals and nitrogen from drainage waters is highly dependent on system size. A small, one MGD unit is projected to cost about \$273.00 per million gallons (\$89.00 per acre foot) for interest and cost retirement, and \$416.00 per million gallons (\$136.00 per acre foot) for Operations and Maintenance, for a total approaching \$700.00 per million gallons of drainage water processed. Systems in the 100 MGD range may cost as little as \$140.00 per million gallons, (\$46.00 per acre foot) and produce as much as \$83.00 worth of surplus energy per million gallons.

A well-designed experimental program involving key laboratory and pilot plant experiments in situ is recommended. To carry out this program will require a research facility consisting of four l-acre high-rate ponds supporting algae harvesting and fermentation systems and laboratory facilities. Such a facility is recommended for further study. Location of the new facility near or at the existing Los Banos Test Facility is recommended.



TREATMENT OF SAN LUIS DRAIN WATER WITH MICROALGAL BACTERIAL SYSTEMS

INTRODUCTION

Selenium, an element analagous to sulfur, other toxic trace elements such as cadmium, chromium, mercury, lead, arsenic, nickel and zinc, originating from sub-surface tile drainage water have been found at high levels, both in certain drainage waters and in residual waters of Kesterson Reservoir in the San Joaquin Valley.

Overt effects on wildlife, such as malformation of coot and duck hatchlings, together with numerous chemical analyses, indicate a genuinely severe environmental problem with selenium and metals resulting from the high levels in drainage waters and the current practice of long-term holding and evaporating these drainage waters. Projections also strongly indicate that, due to eutrophic effects in Kesterson Reservoir, the current problems will accelerate in intensity without remedial action. Closure of Kesterson Reservoir will likely fragment but will not eliminate a problem which demands a rational solution.

Of the many options open for consideration in remedial action treatment of drainage waters is high on the list. The many modes of treatment proposed may be categorized as physical, chemical, biological and combinations of the three. This report deals mainly with the potential of biological treatment with algae and bacteria, employing advanced forms of high-rate, facultative and anaerobic ponds in strategic combinations. The objective is to bring concentrations of selenium and heavy metals in drainage water into conformity with established water quality standards for freshwater aquatic life.

In this report, pertinent literature on microalgal systems in the treatment of wastewater is reviewed, including information on current research and operating systems. The current state-of-the-art on high-rate algal ponds is here described.

1. From the review of available information on ponds and drainage waters, an analysis is presented for the possible use of microalgae in high-rate pond systems to treat agricultural drainage water, particularly the removal of toxic substances such as selenium and other trace elements and the recovery of biomass in energy-efficient systems. The analysis includes possible integration of anaerobic, facultative and high-rate ponds into cost-effective systems as part of the total San Joaquin Valley Drainage Program.

The high-rate pond is herein viewed as the "heart" of any proposed biological systems, because, as a "biomass generator", its' algae fix solar energy more efficiently than any other type of vegetation, creating new organic matter economically from inorganic nutrients in the drainage water. It is likely that this algal organic matter can be used strategically to economically reduce selenate to selenite and that the algae will concentrate, and render removable, heavy metals in integrated ponding systems. Although algae are known to concentrate heavy metals and nutrients, and known to take up small amounts of selenium, the optimum method of designing and operating integrated algal-bacterial ponds for the purpose of removing significant amounts of metals and selenium from drainage waters is, of course, unknown at this time. This desk study is therefore intended to examine some of the potentially feasible algal-bacterial systems, and to suggest further laboratory and field studies on the basis of known facts which appear most promising to yield the information which is needed to design a full-scale system.

- 2. An outline proposal is presented for large-scale pilot plant studies designed to investigate the use and performance in selenium and heavy metal removal from drainage water of integrated microalgal high-rate pond systems alone and in conjunction with facultative and anaerobic ponds. Included is a preliminary outline for a study program with a pilot plant to explore the technical and economical feasibility of these systems.
- 3. Assuming a 1 MGD facility and satisfactory system performance, an estimate is made of the cost of treating drainage water using the proposed method. The cost estimate is then extrapolated to larger systems, using cost trends for sewage treatment plants.

REVIEW OF LITERATURE

Controlled, large-scale microalgae culture in wastewater began after World War II. The impetus for such work was largely due to recognition of the role of microalgae as oxygen generators by Caldwell (1946) and Abbott (1948), although earlier workers,

i.e., Gieseks and Zeller (1939), indicated some recognition of photosynthesis as a significant source of waste oxygenation. Work on mass culture of algae had also been carried out in secret by Japan, Germany, the Soviet Union and the United States during World War II. The work in Japan, Germany and the United States is summarized in Burlew (1953) and in the Soviet Union by Setlik (1970).

In 1950, the United States Public Health Service awarded Professor Harvey Ludwig and the author a grant to study the role of algae in sewage oxidation ponds (Ludwig et al 1952) and, at the same time, the Atomic Energy Commission granted funds to Professors E.W. Steel and E.F. Gloyna of the University of Texas, to explore methods of removing algae from sewage pond effluents. (Steel et al. 1954). In the same time period, the State of California granted funds to Professor George F. Pappenfuss and Paul C. Silva of the University of California, Berkeley, to make a systematic study of the algae in sewage oxidation ponds. (Pappenfuss, 1953). The Ludwig-Oswald, et al, paper indicated that larger concentrations of algae could be grown in the laboratory than out of doors, and that significant sewage treatment was accomplished in the process. Steel and Gloyna found that algae, growing in waste, concentrate almost all radioactive materials, and that the algae can be removed from water by a number of processes. The Pappenfuss and Silva paper indicated that, of the representative ponds, each had similar algal populations. Representative species found in all systems were of the greens genera, Chlamydomonaceae, Oocystaceae, Scenedesmaceae, Euglenaceae, Polyblepharidaceae, Spondylomoraceae and the blue-green order, Oscillatoriales. They found a direct relationship between the number of organisms and the degree of pollution and an inverse relationship between the variety of species and the degree of pollution.

From early 1950 through 1960, work on the growth and waste treatment characteristics of microalgae was continuous at the University of California Engineering Field Station. Although numerous papers resulted from this work, the three major papers related to waste treatment and algae production during that period were "Photosynthesis in Sewage Treatment" (Oswald and Gotaas,1957), "Biological Transformation of Solar Energy" (Oswald and Golueke,1960), and "Wastewater Reclamation Through the Production of Algae" (Oswald, Golueke & Gee,1957). A study on the design of Waste Stabilization Ponds was also produced by Herman and Gloyna, of the Texas Group. (Herman and Gloyna,1958)

"Photosynthesis in Sewage Treatment" (Oswald et al, 1957) established a numerical relationship between algae production and oxygen evolution and set forth a design model for the high-rate ponds based on light energy, BOD and culture density. The paper on "Efficiency" (Oswald, 1960), established the relationship between environmental factors and the efficiency with which algae transform light-energy to cellular energy as a function of CO2 concentration, temperature, detention period, sewage strength and algal species. In the latter case, there was an inverse relationship between efficiency and cell size. Efficiencies in continuous light were found to be between 2 and 6 percent, and to decline with increasing intensity and duration. Efficiency increased with ${\rm CO}_2$ concentration up to about 0.5% in air and then declined. Efficiency also declined with increased detention period and increased with sewage strength. Efficiency declined to near 0 at a temperature of 4°C, and went through a maximum at 20°C, probably due to increased algal respiration at high temperatures. The algae under study in this case were Chlorella. Subsequent studies have shown that each species may have a different optimum temperature for growth. (Grobbelaar et al, 1981).

In the paper "Biological Transformation of Solar Energy" (Oswald and Golueke, 1960), it was shown that microalgae grown on sewage could be subjected to methane fermentation and that 50% or more of the heat of combustion of the algae could be recovered in a mesophilic digester when loadings amounted to 0.125 pounds of algal volatile matter per cubic foot per day. In this paper, there was a detailed analysis of the relationship between channel depth, channel length and energy required for mixing, as well as methane fermentation.

The field study of Oswald, Golueke and Gee, "Wastewater Reclamation Through the Production of Algae" (Oswald et al,1957), conducted at Concord, California, was the first documented effort primarily aimed toward producing, harvesting, drying and utilizing algae from wastewater. Working with a two-acre modified sewage pond during the period 1956 to 1958, they produced more than a ton of sand bed dried algae to be used for animal feeding experiments at Davis, California. It was during that work that the need for mixing shallow, high-rate ponds became apparent. The first mixing was done with a broom and

drag, and later with a motor boat. The latter greatly improved the situation, but was clearly not adequate. This experience led to the concept of flow mixing for shallow ponds, and proved that, in such systems, it is technically feasible to produce algae and to simultaneously treat wastewater to a high degree.

Success in the Concord experiments cited above led to the design of the first mixed waste-fed high-rate pond, which was constructed as an 0.66 acre, 10^6 liter algae production system at the Engineering Field Station of the University of California in Richmond. This unit was designed to operate at depths from 6 to 24 inches, detention periods of 2 to 10 days, and was flow-mixed by four 3-horsepower 1,800 gpm propeller pumps, giving linear flow velocities up to 2 feet per second in an 18-foot wide by 800-foot long channel.

Algae harvesting was accomplished in a 100-gallon per minute continuous centrifuge which produced algal concentrations of one-half to one percent solids. Algae thickening was done in a five-gallon per minute solid bowl centrifuge, which concentrated the algae to 10 to 15% solids. This algae was dried on a steam-heated drum drier, followed by a rotary kiln, which brought the algae to a storable concentration of about 7 to 8% moisture. This algae was transferred to Davis, California, where it was combined with rolled barley and fed to swine, sheep and cattle. This animal feeding was summarized by Hintz et al (1966), who concluded that waste-grown algae plus grain and vitamin $\rm B_{12}$ is equal to meat and bone meal for swine, and equal to or better than soybean oil meal or cottonseed meal for sheep and cattle.

During the decade of the 60's, U.C. Richmond was not the only laboratory working on algae production in wastewaters. Studies by North American Aviation to grow and harvest algae for nutrient removal in waste treatment were also under way at Lancaster, California. Their main thrust was harvesting algae from the Lancaster ponds using aluminum sulfate. This algae was used in extensive chicken feeding experiments. Few systematic reports ever entered the literature from this work, possibly because of proprietary interests. However, site visits to the two 100 M² high-rate ponds (HRP) at Lancaster indicated that they produced dense cultures of microalgae. One problem with

this system was that the propeller pumps used for the HRP were excessively large and produced velocities of 3 to 5 feet per second, which, in light of current experience, requires excessive energy. The high-rate ponds were also fully lined with concrete, making the cost very high.

During the mid 1960's, studies of water reclamation for recreational purposes were also underway at Santee, California, where waste water was impounded after pond treatment. This project was widely publicized, but barely covered in scientific literature. Studies by State and Federal Agencies indicated, somewhat inconclusively, that wastewater reclamation involving primary and secondary treatment followed by ponding and infiltration through several hundred feet of gravel, produced a water which, bacteriologically and virologically, could be acceptable for contact type recreational purposes. Failure to remove nutrients from the wastewater led eventually to a high rate of eutrophication in the reservoirs and, ultimately, the green water dimmed interest in the system.

Another major study initiated by the California Department of Water Resources in conjunction with the United States Bureau of Reclamation and the Federal Water Quality Administration, was pilot plant work to explore the use of microalgae to remove nitrate from subsurface tile drainage water in the Central Valley of California. The studies were conducted in a 1/4 acre high-rate pond and ten .003 acre mini-ponds. Hydraulic loading and nitrate removal were studied. The work, done in the mid to late 1960's, was described in papers by Goldman et al (1969), Arthur et al (1969), Brown et al (1969) and Butterfield et al 1969). The entire study was summarized by Brown (1975). Those studies, in addition to algae production, also involved algae harvesting by natural sedimentation and by coagulation with alum and lime, followed by sedimentation or flotation. Some of the algae were dried on sand beds, for evaluation as animal or fish food. The results of that work indicated that microalgae could, in three to five days, decrease nitrate levels in drainage water from 20 mg per liter to 4 to 5 mg per liter. The problems associated with that study were that the 8-foot by 16-foot mini-ponds from which most of the data were obtained, were mixed only intermittently and at an insufficient velocity. The 1/4 acre high-rate pond, like the Lancaster pond, was

intermittently over-mixed. Thus, the results were not economically favorable. In retrospect, more efficient mixing systems now known, would have greatly improved those systems in performance and economy.

Some of the first work in algae flotation was done in the 1960's by McGarry (McGarry et al (1970)(1973) at the Asian Institute of Technology in Bangkok, Thailand. Since typical sewage was not available there, the studies were designed to produce algae using liquified night soil as the nutrient source. The concept was to produce algae on night soil, harvest with alum using froth flotation, dewater and dry the algae for feeding to chickens. The meager amounts of nitrogen and carbon in night soil plus the high concentrations of alum needed for separation rendered the algae/alum mixture low in nutrient value. This was a disappointing finding of those experiments. An efficient mixing system and the availability of real sewage would have resulted in more favorable findings.

Following the less precise field work, Zabat (1970), Shelef (1972) and Goldman (1974) undertook laboratory -controlled studies of the relationship between nutrient concentrations and algae growth using special laboratory apparatus. The thrust of the work of Zabat was to explore the influence of phosphorus on the growth of Chlorella. He proved that phosphorus is probably never a limiting factor to the growth of algae in sewage or treated sewage-fed bodies of water. Shelef's work on the effects of light, nitrate and ammonia on waste-grown algae productivity was a classic in the field, and the techniques which he developed for his studies were equally applicable for space craft (Shelef et al, (1970). Goldman's work is cited later.

During the mid and late 1960's, three large scale, high-rate, photosynthetic oxygenation ponds were constructed: (1) a 13-acre unit in Concord, California, for domestic waste, designed by Roy Trotter and Associates in consultation with the author; (2) a 19-acre unit by the Spreckles Sugar Company for treatment of beet sugar wastes at Chandler, Arizona, designed by the author; and (3) a 5-acre unit for treatment of domestic sewage at St. Helena, California, by Trotter and Associates in consultation with the author. At

the same time, a 3-acre, integrated pond pilot unit was constructed at the Holly Sugar Company, Tracy, California, for study of the oxygenation of beet sugar wastes. The Chandler system has not been reported in the literature, but the Concord and St. Helena systems were studied in detail by Meron, Zabat and Oswald (1970), and Meron (1970). The Tracy work was also reported in detail by Oswald, Tsugita, Cooper and Golueke (1970). Although these systems were designed as waste treatment units rather than algae production units, they indicated that BOD loadings in the range of 100 to 200 pounds per acre per day were easily accommodated by high-rate ponds. They also indicated that, even though the ponds were 3 feet deep, algae production was on the same order of magnitude as in shallow ponds (100 to 200 lbs. per acre per day). The Chandler systems had galvanized sheet metal dividers (aluminum was originally recommended) and the metal dividers corroded rather quickly, necessitating replacement by earthwork dividers. The St. Helena and Concord dividers leaked and short circuiting occurred. In Chandler, the propeller mixing pumps were designed to discharge underwater, but a change during construction omitted the underwater discharge, resulting in a pump motor overload. Installation of an underwater discharge remedied the situation. The Tracy pilot plant was dismantled within two years, and it was found that their channel dividers leaked underneath and therefore were short circuiting. Jenks and Adamson designed two high-rate ponding systems that had peripheral high-rate ponds; one at Fairfield and one at Modesto, California. The Fairfield plant was studied by Oswald (1974) and found to produce less oxygen than the applied load. In the case of Modesto, the summer cannery load was over 100,000 pounds per day and the 200-acre, 5-foot deep high-rate pond had an oxygenation capacity of only 40,000 pounds per day. If this unit had been shallower, it would have produced more oxygen. Algal growth and harvesting algae from the Modesto system was studied in detail by Ramani (1974)

The next major study of integrated ponds of the St. Helena type was carried out by the author in conjunction with the Napa Sanitation District (Oswald 1975). This 14-month study was the first large scale, controlled study in which two ponds were employed, one a variable and one a control, and detailed information was collected on all aspects of waste treatment. This included BOD, nutrient removal and disinfection, as a function of depth, detention

period and mixing regime. The results of this systematic study clearly showed that integrated ponds provide high degrees of waste treatment, including the oxidation of BOD and the outgassing of ammonia nitrogen, as well as BOD conversion to algal cells. The shallow, systematically-mixed, high-rate ponds apparently conditioned the algal cells in some way that enhanced their settleability, but this was difficult to prove in the Napa system.

The Napa study was followed by a complete, pre-planned test sequence in Manila, the Philippines, with 100 M² pilot plants. (Oswald 1978). The purpose of that study was to explore high-rate ponds for both waste treatment and nutrient removal in a tropical environment. The Manila ponds were the first high-rate waste ponds to be mixed continuously with paddle wheels and the results of that modification gave spectacular results, both with respect to treatment and separation. After several months operation, the cultures developed an autoflocculation characteristic which caused the algae to coagulate and to settle when removed from the continuous mixing field. This resulted in an essentially clear supernatant and high BOD, suspended solids, nutrient and coliform removals (Oswald 1978). This work was summarized by Oswald, Lee, Adan and Yao (1978).

Because of the Manila experience, the 0.6 acre pilot high-rate pond at the University of California Engineering Field Station was divided into two units and each was equipped with a paddle wheel. After a few weeks of mixing at a flow velocity of 10 cm. per second, the contents of the Richmond ponds (0.1 hectares each) assumed properties identical to those in Manila. After withdrawal from the mixing field, the algae coagulated and settled. During mid-summer conditions in Richmond, after withdrawal from the ponds, agglomeration and sedimentation occurred in as little as fifteen minutes. During winter conditions, up to 24 hours were required (Oswald 1980).

The first systematic productivity studies were made in conjunction with the small scale pilot plants operated at Richmond Field Station in 1960 through 1965. In those studies, algal productivities of about 10 grams per meter² per day were obtained with intermittent propeller pump mixing. In the Napa studies (Oswald 1975), productivities ranged from 4 grams per M² per day in winter to 25 grams

per M² per day in summer. Average productivity was about 12 grams per M² per day. In Manila (Oswald,1978), mean productivity was about 13 grams per M² per day, with a peak of 27 grams per M² per day with paddle wheel mixing. In Richmond, with paddle wheel mixing, annual mean productivity of about 15 grams per M² per day, or 55 metric tons per hectare per year, were obtained with a summer peak of 35 grams per M² per day, or 128 metric tons per hectare per year (Oswald and Swanson,1978). These are gross productivities in each case, uncorrected for the organics in wastes introduced. In addition to the University of California studies, algal productivity studies by Wachs and Shelef in Israel (1973) and by Shelef et al (1976), Jerusalem, Elat and Haifa, indicated gross productivities in the range of 20 to 55 grams per M² per day. Shelef ascribed a portion of these high yields to carry-over organic matter in sewage.

During the 1970's, due to the energy crisis, a major portion of the algae work was devoted to methods of producing microalgae and extracting their energy by fermentation processes (Uziel et al, (1975), Benemann (1977) and Eisenberg (1979).

During the 1980's, work continued on methane fermentation of algae (Hall et al, 1981), but also turned to animal waste recovery (Dodd,1980) and to extraction of lipids from microalgae (Shifrin,1980 and Tornabene 1983). After establishment of the Solar Energy Research Institute, funds were advanced for a complete study of shallow mass algal culture systems in Hawaii for production of oils (Laws, 1983, 1984). Laws' studies have shown that by using foils in the channel of high-rate ponds, he has been able to obtain more than twice the productivity of microalgae wt./area/time than he had obtained without foils. The systems are quite costly and energy intensive.

A second ongoing study in Hawaii is that at the Oceanic Institute at Makapuu Point, where two $50~\text{M}^2$ paddle wheel mixed high-rate ponds are employed for the study of production of <u>Spirulina sp.</u> from animal wastes. Spirulina growth on pig wastes was pioneered by Soong in the 1970's, but his papers only became available in the West in current decade (Soong, 1980). No reports

are yet available on the Oceanic Institute work.

A major factor in the development of high-rate ponds in the 1980's has been interest in algae, both as a protein source and for pharmaceutical production. Most of this work has not been published because it is regarded as proprietary in nature by those funding the projects.

However, as a result of recent proprietary projects, the high-rate pond utilizing inorganic media and gaseous carbon dioxide has been greatly advanced toward perfection. Harvesting techniques have also been greatly advanced by those applications. Some of the more promising advanced work has been reviewed recently by the author (Oswald, 1985). Currently, a large high-rate ponding system of 15.5 acres is employed for waste oxygenation at the City of Hollister, California. This unit, designed in 1978 and completed in 1981, has been in satisfactory operation for about 4 years. Used in conjunction with two primary facultative ponds and two settling ponds, the 2 MGD system provides complete waste treatment for less than \$100.00 (1985) per million gallons (Swanson-Oswald, 1978), compared with more conventional treatment processes which cost up to 10 times as much for plants of equal size.

CHARACTERISTICS OF ADVANCED HIGH-RATE PONDS

The advanced high-rate pond is essentially an efficient microalgae growing machine. Early efforts to utilize shallow ponds for algae nutrient stripping were not very successful, because the need for continuous mixing was not understood (Goldman, 1969) and efficient mixing systems did not exist.

It is now recognized that, to attain ideal growth, all shallow outdoor cultures of microalgae must be mixed continuously to avoid cells settling and sticking to the bottom, and to avoid thermal stratification of the water. For example, experience at the Richmond Field Station has shown that unmixed ponds as shallow as 30 cm (12 inches) may have a temperature difference of as much as 8°C from surface to bottom on warm days. Such drastic temperature differences arise when the top few centimeters of unmixed dense cultures of algae absorb and convert most of the sunlight energy to heat. This generally occurs with a

tenfold higher efficiency than the conversion of light to chemical energy in the cells. The warmed surface water tends to remain at the surface because it is lighter than the water in lower strata. Algae growing in this layer utilize and quickly deplete it of available bicarbonate ion and the pH rises rapidly to as much as 11. Under those conditions, calcium carbonate and essential nutrients such as iron and phosphorus tend to precipitate and settle, often carrying the surface algae with them toward the bottom. With clearing of the surface layer, a second layer is exposed to the sunlight and the process repeats itself until an entire unmixed shallow pond may become precipitated. Once algae have reached the pond bottom, they tend to form large flat agglomerations held together by extracellular polymers and bacterial, fungal and bluegreen algal filaments. If light then reaches these layers, they tend to flake off the bottom and rise to the pond surface in mats of varying size, bouyed up by adhering bubbles of photosynthetically released oxygen. These floating mats in turn are swept to the pond edges by wind. Such mats, thickly windrowed at a pond's edge, soon become anoxic and, by day, hot and odorous. They then serve as breeding places for flying insects and may ultimately host large concentrations of Clostridium botulinum, with attendent wildfowl poisoning and death. The nutrient-rich waters of Kesterson Reservoir could cause similar problems, aside from selenium and heavy metals.

CHANNELIZED MIXING

The objectionable chain of events described above, leading to intermittant pond failures, is entirely avoided by continuous mixing. This is best achieved in shallow ponds by channelized flow mixing. A simplified channel layout is shown in FIGURE 1. Basically, the system depicted is an endless raceway through which water is forced to move, essentially like a shallow stream, confined by outside walls and channel dividers. Movement is imparted by a mixing system. Flow mixing has been induced in channelized ponds by propeller or screw pumps, by air lift pumps and by paddle wheels. Properly designed paddle wheels are by far the most efficient and durable high-rate pond mixers, because they involve mainly low-speed moving parts and can be made of corrosion-proof materials. Air lift pumps, while effective, involve air compressors or blowers that tend to wear out in a few years and are less efficient than paddle

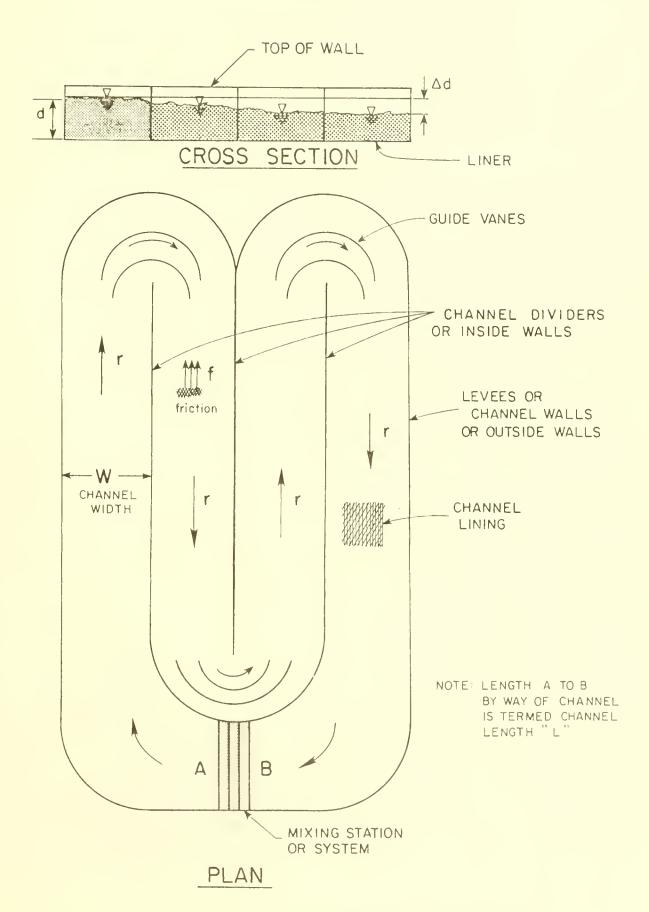


FIG. 1 SIMPLIFIED DIAGRAM OF ENDLESS CHANNEL USED FOR ALGAL CULTIVATION SHOWING NOMENCLATURE OF MAJOR PARTS.

wheels. Also, spargers tend to clog. Ideally, paddle wheels are designed like vane pumps which discharge virtually all of the water entering them, and are thus highly efficient. The paddle wheels shown in FIGURE 2 are ideal for large ponds. Propeller and screw pumps are no longer considered efficient for algal growth systems, although there are several satisfactory systems operating in California - a screw pump system at Hollister and propeller pump system at St. Helena.

MIXING DESIGN

Consider water flowing at depth "d" in a channel with finite width "w" and unspecified length "L". Friction, "f", tends to hinder flow and the area of the channel over which friction acts is termed the hydraulic radius "R". For a 1-foot (0.3 m) length of channel, R is equal to the area of flow divided by the perimeter in contact with water, i.e., for unit length:

$$R = A/P$$
but A = dw
and P = w + 2d
therefore R = dw/ (w + 2d)
(1 b)

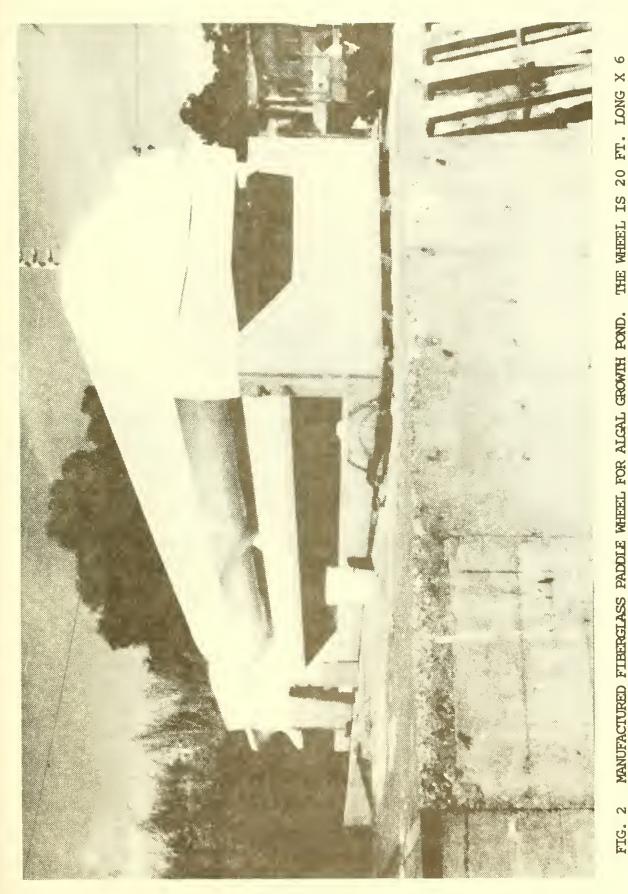
Because hydraulic energy is lost due to friction and because friction increases as the square of the velocity, the depth of flowing water in the channel decreases as a complex function of channel length; that is:

$$\delta d/\delta L = f(v^2, R, f)$$

The Manning equation is a useful empirical solution for these complex functions: $V = (1.486/n) R^{0.67} s^{0.5}$ (2 a)

In equation (2 a), the symbol V represents the mean channel velocity; n, termed the Manning friction factor, represents friction due to channel roughness, R is the hydraulic radius defined above and s is the rate of loss of energy in the channel per unit length, that is 6d/6L.

Rather than precisely evaluate 6d/6L, it is convenient and satisfactory to assume a finite value for the change in depth, $\triangle d$, and to determine the finite length L which corresponds to the assumed change in depth for a given friction factor, hydraulic radius and velocity. To do this, we square both sides of equation (2 a) and solve for s:



REVERSIBLE AND CONTINUOUSLY VARIABLE FROM 0 TO 10 RPM. PHOTO COURTESY HIMSL CRAIG THE WHEEL IS 20 FT. LONG X 6 THE WHEEL IS TURNED BY A HYDRAULIC MOTOR ACTIVATED BY A 3 HP ELECTRICAL MOTOR. THE ROTATION IS IN DIAMETER AND MOUNTED IN AN INVACINATED INSTALLABLE CONCRETE BASE. MANUFACTURED FIBERGLASS PADDLE WHEEL FOR ALGAL GROWIN POND. ENTERPRISES, 4831 MYRTLE DR., CONCORD, CALIFORNIA 94521.

$$s = v^{2}/(1.486/n)^{2} R^{1.33}$$
and since $s = \triangle d/L$ and $R = dw/(w + 2d)$

$$\triangle d = v^{2}/(1.486/n)^{2} [(dw/(w + 2d))] (2 c)$$
and $L = \triangle d(1.486/n)^{2} (dw/w + 2d)^{1.33}/v^{2} (2 d)$

Practical values for each of the factors in equation 2 are discussed below.

CHANNEL ROUGHNESS

The value of n varies with a factor known as relative roughness, related to the mean height of bottom discontinuities compared with the depth of water in a channel. Thus:

$$n = k \in d$$
 (3)

in which & represents the height of discontinuities and d is the channel depth. For example, a discontinuity 1 mm high in a 10 cm deep channel would have a relative roughness of .1/10 = .010. Experimentally determined n values in algal growth channels vary from 0.008 to 0.030, the former for smooth plastic lined channels and the latter for relatively rough earth. By comparing the solution of:

 $\left(1.486/n\right)^2$ for n = 0.010 (22,082) and n = 0.030 (2,453) it is apparent that, other factors equal, n has a profound effect upon the permissible channel length for a given Δd . The author's estimates of mean values for n using various materials for channel liners are shown in TABLE 1.

CHANNEL VELOCITY

Experience with large scale algal cultures indicates that fluid velocities as low as 5 cm per second will prevent thermal stratification and maintain algae or algal-bacterial flocs in turbulent suspension. In wide, shallow channels, however, it is not possible to maintain uniform velocity across the channels, particularly at bends, and accordingly minimum linear velocities of about 15 cm (1/2 foot) second are necessary to obtain at least 5 cm second (0.16ft sec 1) in most locations. Because, as shown below, the power required to mix cultures increases as the cube of the velocity, it is worthwhile to minimize velocity whenever energy is a major cost factor. Velocities greater than 30 cm/sec (1 foot/sec) will result in large values of \triangle d in long channels and may

Material	Manning's n*
smooth plastic on smooth concrete plastic with "skrim" on smooth earth smooth plastic on granular earth smooth portland cement conrete smooth asphalt concrete coarse troweled concrete, rolled asphalt gunnite or sprayed membranes composted smooth earth rolled coarse gravel, coarse asphalt	.008 .010 .012 .013 .015 .016 .020
rough earth	.030

^{*}Based on experience with wide shallow channels about 1.3 ft. (0.4m) deep

require high channel walls and higher divider walls that add to pond cost.

CHANNEL DEPTH, WIDTH AND AREA

Channel depth should be based on the concentration of algae absorbing light. Light penetration is easily measured in a culture by visual observation of the extinction of a black and white disk submerged in the pond (Secchi disk). This measured extinction depth, d_s , is about 1/2 the actual depth of sunlight penetration, dp, because light must enter and strike the disk and then exit to be observed.

Experimental observations (Oswald,1978) indicate that in outdoor cultures d $_{\rm S}$ and d $_{\rm D}$ are related to the culture concentration approximately as:

$$d_s - 3000/C_c$$
 (4 a)

$$d_{p} = 6000/C_{c}$$
 (4 b)

in which $C_{\rm C}$ is the concentration of green algae in mg liter⁻¹, d_s is the Secchi depth in cm, and d_p is the total depth of light penetration in cm. Field observations of the high-rate pond in Richmond further indicate that culture concentration in light-limited continuously mixed cultures approaches that which permits light to penetrate only 2/3 of the actual culture depth. Accordingly, d_p = 2/3d and:

$$C_c = 1.5 \times 6000/d = 9000/d$$
 (4 c)

As an example, with sufficient nutrients and permissible temperature, a continuously mixed outdoor culture being operated at 30 cm depth may obtain an average maximum light-limited algal concentration approximating 9000/30 = 300 mg. liter⁻¹. This would only be slightly greater during periods of high light intensity and slightly lower during periods of low light intensity because the penetration of light is proportional to the log of its intensity. A corrolary to this is that high concentrations of algae can only be attained out of doors by using shallow culture depths. Major disadvantages of shallow depths are that cultures tend to overheat on hot days and, as will be illustrated in the following section, are more difficult and expensive to mix.

MIXING DEPTH RELATIONS

To illustrate the interrelationships of depth and mixing requirements, consider now equation (2d) for several depths - say 1, 2 and 3 feet (0.3, 0.6 and 0.91 meters). For this exercise, we will assume a channel width of 40 feet (12.2 m), a mean velocity of 1/2 ft/sec (0.15 m/sec), and an n value of 0.025, reflecting a relatively rough surface. \triangle d is made equal to 3 inches (0.25 ft.)(.076 m) because such a differential head, or actually more, can be maintained by paddle wheels of the type shown in FIGURE 2.

Substituting these values in equation (2d) for 1, 2 and 3 feet (0.3, 0.6 and 0.91 m) depths, one obtains:

```
For d = 1 ft. (0.3 \text{ m}): L = 3,310 \text{ ft. } (1,009 \text{ m})

For d = 2 ft. (0.6 \text{ m}): L = 7,822 \text{ ft. } (2,384 \text{ m})

For d = 3 ft. (0.9 \text{ m}): L = 12,648 \text{ ft. } (3,856 \text{ m})
```

Thus, for the same head loss of 0.25 ft (0.1 m) a channel 3 ft. (0.9 m) deep can be 3.8 times as long as a channel 1 ft. (0.3 m) deep.

AREA

One can then integrate width and length to determine the area mixed.

$$A = WL \tag{5}$$

in which A is the area, W the width and L the channel length (See FIG. 1).

For 1 foot (0.3 m): A = 40 x 3,310 = 3.04 acres (1.23 ha).

For 2 foot (0.6 m): A = $40 \times 7,822 = 7.18 \text{ acres}$ (2.91 ha).

For 3 foot (0.91 m): A = 40 x 12,648 = 11.61 acres (4.7 ha).

Areas can be made larger by increasing channel width and mixing with paddle wheels side by side or by using paddle wheels in series. Channel length between paddle wheels in series would be limited by \triangle d, which could be as much as 6 inches for wheels of the type shown in FIGURE 2.

CHANNEL CHARACTERISTICS

The importance of smooth channels can be illustrated by determining areas that

could be mixed with a single paddle wheel in a 40-foot channel if the channel n value were 0.015 rather than 0.025. For n = $0.015, (1.486/n)^2$ becomes 9,814 instead of 3,533 and the channel lengths would be: for 1 foot, 9,195 ft.; for 2 feet, 21,735 ft.; and for 3 feet, 35,135 ft. The corresponding areas for a 40-foot width would be 9.01 acres (3.64 ha), 19.95 acres (8.08 ha) and 32.26 acres (13.06 ha). Actually, channel lengths should be slightly less than indicated by the Mannings equation because, with each complete reversal in flow direction, there is a loss of kinetic energy of approximately $V^2/2q$. For a velocity of 0.5 ft sec-1, this amounts to about .0038 feet (.001 m) per reversal. Accordingly, one must determine the number of reversals in direction in a raceway before the hydraulic design can be completed. For the channel shown in FIGURE 1, the number of reversals is 4 (equal to the number of parallel channels). Accordingly, the kinetic energy loss in the raceway would be $4 \times .0038 = 0.015$ ft. (.004 m). This would need to be subtracted from the available head $(\triangle d)$ in equation (2d) to determine actual permissible channel length. Since a total/ Δd in equation (2d) of .25 feet was assumed, and the kinetic loss is 0.15, we have 0.25 - .015 = .235 ft (0.05 m). By proportion, the channel area would then be diminished to $.235/.25 \times 7.18$ acres = 6.73 acres (2.22 ha), a decrease of about 6% in area. The corrected areas for the several ponds are: for 1 foot; 2.85 ac. (1.15 ha); for 2 feet, 6.74 acres (2.73 ha) and for 3 feet, 10.41 acres (4.42 ha).

POWER REQUIREMENTS

To accomplish the channel mixing, power input to the mixing system can be determined on the basis of the total \triangle d including friction and kinetic energy losses, and the quantities of water in motion.

$$HP = QW \triangle d/550 e \tag{6}$$

in which HP is horsepower, Q the quantity of water in motion in ft^3 second⁻¹, W is weight of water in lbs. ft^{-3} , $\triangle d$ the change in depth in ft., ε the efficiency of the paddle wheel and the constant 550 has the units ft-lbs second⁻¹ HP⁻¹.

For a channel width w and depth d, the quantity of flow Q in ft^3 sec⁻¹

$$Q = wd V (7)$$

in which w and d are expressed in ft. and V is in ft. sec. $^{-1}$. As an example,

for a 40-foot wide channel 2 feet deep flowing at a velocity of 0.5 ft - \sec^{-1} with a \triangle d of 0.25 feet, the power required assuming an efficiency of 0.5 is:

 $HP = 40 \times 2 \times .5 \times 40 \times 2 \times .5 \times 62.4 \times 0.25/550 \times 0.5$

HP = 2.26 HP

and since 1 horsepower = 0.746 kilowatts:

kw = 1.68

for 24 hours the power consumption would be:

 $1.68 \times 24 = 40.29 \text{ kw hrs day}^{-1}$

thus a 6.73 acre x 2 feet deep raceway pond could be mixed continuously for only $40.29 \text{ kw hrs day}^{-1}$. This amounts to only $40.29/6.73 = 5.97 \text{ kw hrs acre}^{-1} \text{day}^{-1}$ (18.25 kw hrs ha⁻¹ day⁻¹).

To illustrate the problems arising from higher velocities, we return to equation (2d) and determine the hydraulically permissible channel length involved in a velocity of 1.0 ft. sec. (30.4 cm. \sec^{-1}) for a channel 2 feet (20 cm) deep. Again, assume a loss of $V^2/2g$ at each of 4 reversals of flow for 1 ft/sec. This amounts to .062 feet which must be subtracted from the available head of 0.25 feet, thus 0.25 feet - .062 = .188 feet.

 $L = (.188) (1.486/.025)^{2} \left[40 \times 2/40 + 2 \times 2\right]^{1.33}/(1)^{2}$

L = 1,471 feet (448 m)

 $A = 1,471 \times 40 = 1.35 \text{ Acres} = 0.546 \text{ ha}$

 $HP = wdv \times W \times \Delta d/550 e$

 $HP = 40 \times 2 \times 1 \times 62.4 \times 0.25/550 \times 0.5 = 4.54 HP = 3.35 kw$

or, operating 24 hours day⁻¹, 80.6 kw hrs day⁻¹ are required. Because the area mixed only 1.35 acres, the energy required is 0.6/1.35 = 59.7 kw hrs acre⁻¹ day⁻¹. Thus, in going from a channel mixing velocity of 0.5 feet sec⁻¹ to 1.0 feet sec⁻¹, we have limited culture area and increased the energy required to mix an acre of culture 2 feet deep nearly 10 fold. Actually, in doubling the velocity, the power required should increase 8 fold (2^3) .

Clearly, with respect to energy savings, one should design ponds to operate

at the minimum velocity that prevents algal sedimentation and pond surface stratification and overheating. This velocity may only be determined from a local pilot plant. Variable speed paddle wheels of the type shown in FIG. 2 permit day-by-day adjustments of velocity based on temperature and experience.

PRODUCTIVITY

If one knows the concentration of algae actually attained in a given waste and also the hydraulic and cell residence time and the culture depth, the production of cells material per unit of area and time can be calculated using the rational formula:

$$P = k dC_{c}/\theta$$
 (8)

in which P is the productivity, d the depth, $C_{\rm C}$ the cell concentration and θ the residence time. If cell concentration is measured in mg/liter⁻¹, d in cm, and mean cell residence time in days, and P is to be expressed in grams m⁻²day⁻¹, then the value of k for algae is 0.01 and for 0xygen .015. If d is expressed in meters and again cell residence time is in days, $C_{\rm C}$ is in mg/liter⁻¹ and P is in grams m⁻² day⁻¹ the value of k for algae is 1.0, and for $O_{\rm C}$ is 1.5.

As an example, a pond operating at steady state with a depth of 30 cm (0.3 m), a mean cell residence time of 4 days and a cell concentration of 300 mg/liter will have a productivity:

$$P = .01 \times 30 \times 300/4 = 22.5 \text{ grams meter}^{-2} \text{ day}^{-1} \text{ algae, or } 33.75 \text{ gm m}^{-2} \text{ day for } 0_2$$

or:
$$P = 1 \times 0.3 \times 300/4 = 22.5 \text{ gm meter}^{-2} \text{ day}^{-1} \text{ algae, or } 33.75 \text{ gm m}^{-2} \text{ day for } 0_2$$

Since there are 365 days in a year, $10,000 \text{ m}^{-2}$ hectare⁻¹ and 1,000,000 grams in a metric ton, a productivity of 1 gram m⁻² day⁻¹ is equivalent to:

1 x 10,000 x 365/1,000,000 = 3.65 metric tons per hectare per year Similarly, since there are 4,047 m² acre⁻¹, 454 grams pound⁻¹ and 2,000 pounds short ton⁻¹, a productivity of 1 gram m⁻² is equal to:

 $1 \times 4,047 \times 365/454 \times 2,000 = 1.63$ short tons per acre per year.

Using these conversion numbers, the above productivity of 22.5 grams of algae m⁻² day⁻¹ sustained for a year would equal 82.1 metric tons ha⁻¹ year⁻¹ or 36.6 short tons acre⁻¹ year⁻¹ of algae. Using 1.5 times as much oxygen, the productivity is 123 metric tons ha⁻¹ year⁻¹ and 55 short tons acre⁻¹ year⁻¹ for algae. Such productivities are actually attainable in properly located, designed and enriched systems.

LIMITATIONS ON PRODUCTIVITY

If depth, cell residence time and culture concentration are accurately known, productivity can be precisely determined from equation (5b). One might assume then from equation (5b) that, to increase productivity, one should increase depth or decrease cell residence time. The reality, unfortunately, is that equilibrium cell concentration decreases with increasing depth (usually more rapidly than depth is increased - due to extraneous turbidity) so increases in productivity resulting from increased depth are very limited (although mixing may be facilitated). By the same token, if cell residence time is shortened by decreasing the hydraulic residence time of the culture liquid, the cell concentration tends also to decline due to dilution so again, an increase in productivity may not occur. One is therefore left with the conclusion that predictive equations, although rational, are limited in value and there is a need to carry out local experiments to determine in situ the influence of depth and cell residence time on culture concentration and productivity of cells and oxygen.

With regard to the current status of localized experiments, the author has conducted or is familiar with several systematic studies of productivity that have been made on relatively large outdoor systems in several parts of the world - specifically, Napa, California; Manila, Philippines; Haifa, Israel (Shelef) and Richmond, California. In each case, when observed productivities were plotted against solar energy input, the results proved to be similar to the model shown in FIGURE 3. The general empirical equation for observed productivity is thus:

$$p = k_p (S - S_0)$$
 (9)
in which p represents productivity in grams $m^{-2} day^{-1}$, S is the total solar

In the engineering of algal cultures, a linearized model of productivity vs. solar energy (curve 2 in FIGURE 3) is more useful that the idealized model (curve 1 in FIGURE 3), because the lowest productivities indicated by the idealized curve are those for which some factor other than light becomes limiting to growth and should be avoided, if possible.

In TABLE II, linearized data are tabulated for the 4 relatively large pilot ponding systems noted above. These are shown graphically in FIGURE 4. As indicated in TABLE II, the systems are not strictly comparable because a number of factors differ, specifically water temperatures, culture cell residence times, depths and mixing regimens. Chronologically, the Napa experiments preceded the Manila experiments, the Haifa and Manila experiments were approximately concurrent and the Richmond experiments were last; consequently, at Richmond it was possible to take advantage of the knowledge gleaned from the others. Although the Haifa experiments attained higher average photosynthetic efficiency than Manila, they required much more energy for mixing. The low productivity coefficient and efficiency in Napa are attributed to both the 60 cm depth and the longer cell residence time of 6 days. There, cell concentrations never exceeded 120 mg liter ⁻¹ and daily cell yield was thus less than 20 mg liter ⁻¹ day ⁻¹ (12 grams m⁻² day ⁻¹).

All factors considered, the conditions of depth, residence time and mixing indicated for Richmond in TABLE II are most advantageous, since they attained the highest productivity coefficient and efficiency with the lowest So.

A rational correlation of S_0 with temperature is indicated by the available data. Both Richmond and Manila had equal depths and residence times and a similar mixing regime, but differed in mean temperature by 13° C and in S_0

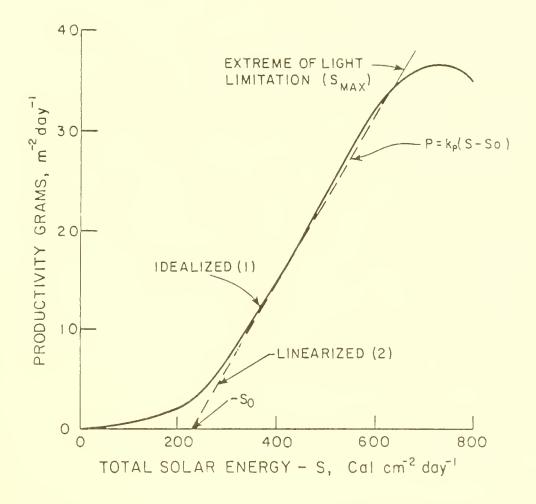


FIGURE 3 IDEALIZED AND LINEARIZED MODEL OF THE INFLUENCE OF SOLAR ENERGY ON ALGAL PRODUCTIVITY IN A CONTINUOUSLY MIXED LIGHT LIMITED SHALLOW POND

TABLE II

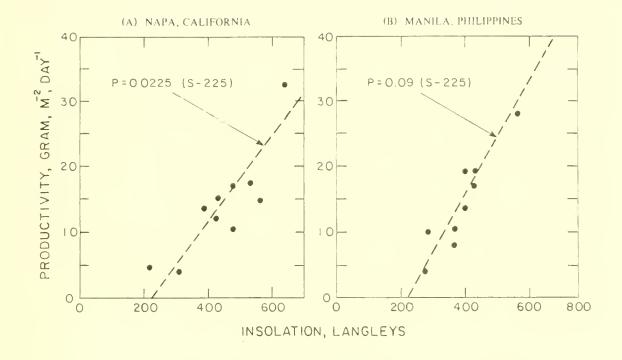
PRODUCTIVITY OF MICROALGAE
GROWING ON DOMESTIC SEWAGE IN HIGH RATE PONDS

Geographic Location	Napa Calif(1)	Manila <u>P.I.</u> (I)	Richmond Calif(1)	Haifa Israel(2)
Culture Area ha	0.1	.01	0.1	.05
Mean Culture Depth, cm	60	30	32	37.5
Mean Water Temp °C	16.5	28.6	15.6	17.5(3)
Mean Cell Residence Time, days	6.0	4.0	4.0	3.14
Mixing Method	pump	paddle wheel	paddle wh	eel brush
Mix Time hrs/day,0, night, N fast slow	1/D,2.5N	2N 22D	0 24	1 2N 1 2D
Mix Velocity Cm sec ⁻¹ fast slow	30 -	30 5	12.5	30 15
Solar Energy S _o cal cm ⁻² day-1	225	225	125	138
Maxium Solar S_m cal cm^{-2} day-1	650	600	500	653
Productivity Coefficient kp	.0225	0.090	0.108	0.070
Mean Photosynthetic Efficiency %	1.1	2.12	3.56	2.84

^{1.} Author's data

^{2.} Shelef: (1980)

^{3.} Estimated from World Atlas



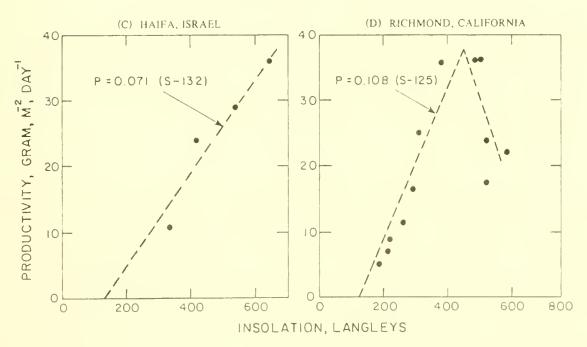


FIG. 4 ALGAL PRODUCTIVITY IN VARIOUS GEOGRAPHICAL LOCATIONS. (SEE TABLE II FOR DEPTH, RESIDENCE TIME, & MIXING REGIMES)

by 100 calories cm⁻² day⁻¹. The increase in S_0 with temperature was therefore about 7.7 cal cm⁻² day⁻¹ C^{0-1} . S_0 is also clearly influenced by other factors. For example, Napa, although much cooler than Manila, had an S_0 equal to Manila's (225). Napa had a depth of 60 cm, a residence time of 6 days and mixing at 1 foot/second, but only for a small fraction of each day.

Each of these factors apparently tended to increase \$\sigma_0\$ but the special experiments required to arrive at more than empirical models for predicting the interacting influences of variable depth, residence time, temperature and mixing regimes on threshold solar energies and productivities remain to be done. For the present, as noted before, the Richmond regime of 30 cm depth, 4-day residence time, liquid organic waste as nutrient and continuous flow mixing with paddle wheels at 12 to 15 cm sec⁻¹ appears to be near optimum in maintaining sunlight as the limiting factor and thereby maximizing both efficiency and productivity at minimum cost in outdoor cultures.

ALGAE SEPARATION

In waste ponds discharging more than 2 million gallons per day of liquid containing more than 20 mg per liter of suspended solids, including microalgae, Federal and State requirements demand algae removal. Special studies of algae separation have been reported in the literature for more than 35 years, and reviews of progress have appeared from time to time (Golueke et al 1968) and (Shelef, 1984).

Aside from their relatively minute concentrations in water, algae are difficult to harvest because they have a density just slightly greater than that of water and because they have a strongly negative charge on their surface. When cell residence time is short and the cells are in a logarithmic growth stage - as is desirable for high productivity - their negative surface charge is very high - and difficult to neutralize so the cells tend to remain dispersed. On the other hand, when the cell residence time is longer and only a relatively few cells are dividing, there is a decrease in the mean negative surface charge and the cells may actually tend to clump and settle in a phenomenon called autoflocculation. Not only do older cultures have reduced surface charges, but Pavone et al (1974) have demonstrated excretion of organic polymers by older

cultures, polymers that can actually be used to harvest the algae producing them and other algae as well. In this case, gentle stirring such as with paddle wheels may be all that is required to effect flocculation. Many of the planktonic microalgae will grow attached to surfaces if given an opportunity. The excretion of attaching filaments has been demonstrated by several authors, summarized by Hall (1985)and Nurdogan (1985). The latter authorities have also shown that in algal-bacterial cultures, clumping and intertwining of bacterial and algal extracellular fibers is commonplace. It appears, however, that in taking advantage of the natural clumping and settling phenomena, one may give up much in the way of productivity. Since disperse rapidly-growing cultures are most productive and older, less productive cultures are easiest to harvest, there clearly must be a trade-off optimum cell residence time in which loss of productivity is offset by decreased cost of harvest. Local pilot systems involving both growth and harvesting of the desired algae should therefore be employed to determine for each season, the cell residence time at which algal removal cost is least, and productivity is greatest.

HARVEST BY COAGULATION, SETTLING AND FLOTATION

The technique of coagulating and settling suspended materials such as iron with lime has been known for at least a hundred years, and its use to remove suspended solids such as algae from community water supplies began with the advent of rapid sand filtration about 1920. For rapid sand filters to function properly, some carry-over flocculent material called "floc" is needed. The most effective coagulants to flocculate algae are aluminum sulfate, $Al_2(SO_4)_3$ and ferric sulfate, $Fe_2(SO_4)_3$. Aluminum chloride and ferric chloride are also effective. Typical reactions are:

$$A1_2(S0_4)_3 + 3Ca(HC0_3)_2 \rightarrow 3CaS0_4 + 2A1(OH)_3 + 6C0_2$$
 (10)

$$Fe_2(SO_4)_3 + 3Ca(HCO_3) \rightarrow 3CaSO_4 + 2Fe(OH)_3 + 6CO_2$$
 (11)

$$2A1C1_3 + 3Ca(HCO_3)_2 \rightarrow 3CaC1_2 + 2A1(OH)_3 + 6CO_2$$
 (12)

$$2FeCL_3 + 3Ca(HCO_3)_2 \rightarrow 3CaCl_2 + 2Fe(OH)_3 + 6CO_2$$
 (13)

In each case, the metallic hydroxides are insoluble and tend to clump together, entrapping and enmeshing algal cells while they settle or float.

Fresh $\operatorname{Ca(HO)}_2$ is also effective in producing an algal floc with, probably CaCO_3 :

$$CaO + 2H_2CO_3 \rightarrow Ca(HCO_3)_2 + H_2O$$
 $CA(HCO_3)_2 + CaOH \rightarrow 2CaCO_3 + 2H_2O$
(14)

Settling of particles occurs approximately in accordance with Stokes Law.

Flotation of the particles can be enhanced by entrapped gases such as the released CO_2 or O_2 or by dissolved gases compressed in the water and then released (dissolved gas flotation). Generally, as pointed out by Parker (1978), algal particles can be made to float upward much more rapidly than they settle downward, so that flotation tanks can be designed much smaller than settling tanks. It is also generally possible to remove the surface material called "float" more completely than settled material, which is so light is is difficult to remove from the bottom of a settling tank. Algal float will also generally have a much higher waterfree solids content than algal sludges. Studies of algal coagulation and flotation have been conducted with alum and various cationic polymers at Sunnyvale, California for a number of years (Farnham 1980).

Shelef and Sandbank (1976) have described the process of electroflotation in which small bubbles of hydrogen are generated by electrolysis. Because hydrogen is light and insoluble, it floats to the surface carrying flocculated algae along with it. Except for the method of producing bubbles, this process is similar to dissolved air flotation. Salerternick et al (1974) have produced fine bubbles for flotation of algae using nozzles which they claim as effective as dissolved oxygen.

HARVEST BY SCREENING

Some filamentous microalgae, such as <u>Spirulina</u> sp, are easily removed from their liquid media on vibrating, oscillating or cascade screens because their filaments bridge the screen openings and the media can drain away to be discharged or returned to the growth system. Sekoulov (1979) has reported that coarse screening of an upflow stream, containing algae with an adjusted pH

accomplishes highly efficient algae removal in waste effluent streams.

The finer screens (50 to 100 micron openings) are highly effective for Spirulina sp., but screens with openings greater than 100 microns capture only a fraction of the filaments. Microgreen algae such as Chlorella sp. and Scenedesmus sp. pass through 50 micron screens with no removal. Openings of about 5 microns will retain most Chlorella, and openings of 20 microns will retain most Scenedesmus. However, these screens have a low throughput rate often less than 10 liter m⁻² min⁻¹ and, like filters, must be continuously backwashed to prevent clogging. The tendency for fine screens to clog resulted in development of various types of continuous screens such as the rotary microstrainers (Kormanik et al, 1978) and the ingenious continuous screenbacked filters developed by Dodd (1972).

HARVEST BY FILTRATION

Many types of filters have been used to harvest algae, but all suffer the universal problem of rapid clogging. Moving filters that can be continuously backwashed are therefore required. These have limited throughput rates, and the accumulated solids are necessarily diluted by the backwash water. Rotary vacuum filters suffer the same fate. Automated thin wall sand filters (Arndt 1971) have shown some promise since the entire cycle of filtration and backwash can be automated and activated as frequently as needed.

Conventional slow sand filters require frequent surface cleaning and it is difficult to separate product from sand. Rapid sand filters only work if algae are first coagulated and flocculated. The filters then must be backwashed frequently and for prolonged periods. As a consequence, concentration factors of only 10 to 20 may be attained reliably with rapid sand filters. Precoated diatomaceous earth or celite pressure filters have been used to remove algae from swimming pool water and for emergency drinking water supplies since World War II, but again, clogging is rapid and concentration factors are meager when this type of filter is applied to capture algae in high-productivity cultures. Pressure sand or candle filters fare little better, while vacuum filters clog almost immediately, and can only be used with the moving belt or rotating cylinder principle – an expensive problem in high

concentration cultures.

HARVEST BY CENTRIFUGATION

Almost any species of microalgae can be removed from their media by centrifugation in the range of 500 to 5,000 times gravity ("g"). At 500 x g,10 minutes of residence time may be required in a solid bowl centrifuge to form a pellet of algae, whereas in a disk centrifuge at 5000 g, residence time may be only a few seconds, but concentration factors may be only 10 to 20 times the input and removals may be only fractional. In their extensive studies of algal centrifugation, Golueke et al (1965) concluded that, to be economical for algal recovery, very large (circa 84 inches [213 cm] diameter) 10,000 liter min continuous disc centrifuges would be required. Such large centrifuges are not commercially available at this time, and smaller 30 inch (76.2 cm) disc centrifuges require more than 3500 kw hrs to separate a ton of dry algae from 200 mg liter suspensions at a throughput of about 1000 liters per minute.

Vertical solid bowl centrifuges have meager throughput rates. For example, in the author's experience, a 48-inch (122 cm) solid bowl centrifuge has a permissible throughput rate of only 150 liters per minute when harvesting 15 micron diameter microalgae at 3000 g. Horizontal solid bowl centrifuges do not separate microalgae without coagulation and precoating, so they are of little value over other concentration systems that cost less.

PHOTOTACTIC SEPARATION

Much interest and some effort has been placed on applying to separation the fact that motile microalgae tend to move toward or away from certain wave lengths and intensities of light (Miller and Wilke, 1972). Thus far, laboratory demonstrations of this phenomenon have been successful but erratic. Large scale systems have not been attempted for lack of design criteria. One problem is that suspensions of algae of economical concentrations absorb light so strongly that only a very thin layer of cells could be subjected to the correct intensity for phototactic attraction or repulsion at any instant. Based on current information, the engineering design of a 1000 liter per minute phototactic separator would likely involve hundreds of square meters of controlled

illumination and consequently, to be economical, applications would require a most exotic and valuable product. It is doubtful that phototactic separation will be able to compete with flocculation and flotation, but, if an additive-free product is required, it might compete favorably with centrifugation. Large pilot scale installations will be required, however, before one can evaluate the probable efficiency and cost of a practical system.

BEST PRACTICABLE TECHNOLOGY

The bottom line at the present is that autoflocculation, using small amounts of lime with in-pond subsidence into special pits (Oswald, 1977) is economical but not highly efficient. Autoflocculation with dissolved air flotation (DAF) is more efficient, but also more costly (Nurdogan, 1985). Chemical coagulation with dissolved air or electroflotation is the most reliable and fastest process currently available. Innovative short residence time DAF units of the type developed by Krofta (1983) may also prove very economical. Selection of a coagulant for use with DAF units must be based on local conditions and needs. For example, experience has indicated that algae grown in the drain had a strong tendency to settle when mixing was withdrawn. (Butterfield, 1969). Accordingly, local experiments with properly designed high-rate ponds will be required to determine the efficiency and cost of harvesting with innovative DAF and other types of harvest systems.

THE HIGH-RATE POND IN SELENIUM REMOVAL

The available evidence that algae growing in drainage waters will directly remove significant amounts of selenium is inconclusive. The reason for this statement is that the meager data on selenium uptake by algae in Central Valley drainage water (see TABLE III) does not include such essential facts as:

- o type of algae involved
- o other organisms present
- o time of contact
- o growth rate and residence time of the algae
- o physical conditions during algal water contact, such as depth, light, temperature and mixing
- o coexistent physical water quality characteristics, such as pH, Eh and dissolved oxygen
- o the total dissolved solids and their major components in the specific water

CONCENTRATIONS OF SELENIUM REPORTED IN MICROALGAE
GROWN IN CENTRAL VALLEY DRAINAGE WATERS

TABLE III

Genera and Source	Reported Concentrations Mg Kg ^l	Reference		
Filamentous, Algae				
Kesterson	12.5 - 245.9	Saiki, 1984		
Volta	0.3 - 0.9	11		
"Plankton"	0.0			
Kesterson	68.3 - 107.7	11 11		
Volta	1.5 - 2.9	n II		
Marsh Ponds (1985)	30.0 - 50.0	Beck, 1985		
Mixed Green microalgae				
Firebaugh (1968)	5.33 (1)	Westberg, 1985		
Preserved dry by				
Jim Arthur	1.5 (1)	H n		
Mixed Green Algae	(2)	McKeown		

⁽¹⁾ Same sample

⁽²⁾ Data Pending

- o the trace mineral analysis of the specific water
- o the nutrient content of the specific water

There is evidence that each of the factors listed are determinant in high-rate ponds, and each can play a significant role in the efficiency of selenium and heavy metal removal by algal bacterial systems. Each of these factors is briefly considered in the following paragraphs.

TYPE OF ALGAE

The influence of Class, Order, Family, Genera or Species of algae on uptake of trace minerals is lacking in the available literature. In fact, in major reviews of algal systems such as the "Handbook of Microbiology", Vol. II, covering fungi, algae, protozoa and viruses (Laskin et at, 1977); "Algal Biomass" (Shelef et al, 1980); "Wastewater for Aquaculture" (Grobbelaar, 1981), there is barely any mention of algal trace mineral content.

The micro-nutritional requirements of microalgae in media have been developed by trial and error, using bioassay techniques and generally species differences are limited to "fresh water algae" and "salt water algae" (Stein 1973), but analyses of the resultant algae are apparently lacking in most cases. An exception is the health food blue-green algae <u>Spirulina</u> which, because it is sold as a human food, has been analyzed for trace minerals including selenium, variously reported to be 6.2 mg/kg, 5.5 mg/kg (Switzer, 1982) and 0.4 mg/kg (Hills, 1980). This <u>Spirulina</u> is washed and spray dried so that absorbed ions are likely removed to some extent.

From the above, it is apparent that more studies of the trace mineral content of drainage algae should be done, particularly with respect to the priority pollutants arsenic, cadmium, chromium, copper, lead, mercury, selenium and zinc. It should not be assumed that integration into the tissue of algae is the only method by which trace substances are removed by algal systems, and by particular species. Two other highly effective mechanisms exist which are associated with rapidly growing algae: ion adsorption and compound precipitation. Adsorption is due to the highly negative charge on the surface of rapidly

growing algae and precipitation is due to increased pH, always associated with rapid algal growth in high-rate ponds under outdoor conditions.

OTHER ORGANISMS

The interaction of algae with fungal and bacterial systems in the natural environment has been known to both enhance and harm algal growth. Enhancement results from the release of carbon dioxide and ammonium resulting from bacterial and fungal action, and a stimuation of algal division due to release of growth factors such as indolacetic acid and vitamin B_1 . Also, on the positive side, protozoans, rotifera and ostracods, which graze on algae, may cause multiple crops of algae to grow during a short time. Fecal pellets from these grazers usually contain large numbers of intact algae which, although alive, cannot escape from the pellet, and thus end up settling to the bottom, carrying with them adsorbed ions.

On the down side, predators can decimate an algal population by overgrazing and thus depriving themselves of food. Experience has shown that much of this type of phenomenon is seasonal, and that, if cultures are maintained under good conditions, the predator -prey "overshoots" dampen out with time and virtually disappear except for seasonal "flare-ups". Recent work at the University of California Engineering Field Station high-rate ponds indicate that minute doses of rotenone and other low-toxicity pesticides will control large microalgae predators such as daphne and cyclops. Even these, however, seem to have less severe effects in mature continuous cultures in high-rate ponds.

TIME OF CONTACT

In some of the earliest studies of microalgal kinetics at the University of California Field Station, the author exposed a fast-growing culture of mixed Chlorella and Scenedesmus to minute amounts of uranyl acetate $(UO_2 (C_2H_3O_2)_2)$ for about a minute and then centrifuged the culture to separate the algae from the supernatant. A Geiger counter was used to determine the activity in each fraction. The results showed that 99.9 percent of the radioactivity was in the algae fraction and 0.1 percent was in the

supernatant. The same uranyl acetate solution without algae addition gave an even distribution of activity after centrifutation. The unmistakeable conclusion of this experiment was that the uranyl (and perhaps the acetate) ion was almost instantly adsorbed to the algae cells.

In studies of the Napa Sanitation District's waste disposal system, the author found that, in addition to domestic sewage, the district received wastes from two tanneries, discharging significant amounts of chromium in both hexavalent and trivalent forms. The Napa system has four (4) large ponds in series, and is provided with recirculation from the fourth pond into the first pond for seeding and load distribution. Chromium determinations made on influent (1500 μ g/l) and each pond in series (see FIGURE 5) showed that overall, more than 97 percent of the influent chromium was removed, most of it in the primary pond. The chromium was accounted for in terms of its content in bottom sludges. Since chromium is not a normally large constituent of algal cells, one must conclude that the strongly positive ${\rm Cr}^{3+}$ and ${\rm Cr}^{6+}$ ions were adsorbed to the negatively-charged algae and settled with them into the bottom sludges.

Again, the almost instantaneous affinity shown by algae for Fe⁺⁺, Fe³⁺ and Al³⁺ ions in flocculation is further evidence of their adsorptive capacity to polyvalent cations. Finally, Wang et all (1984) have examined the "bioaccumulation" of nickel by several species of microalgae and found concentration factors of greater than 10³ by Scenedesmus, Synechococcus, Oscillotoria, Chlamydomonas and Euglena, all common inhabitants of waste ponds. Although nickel⁺⁺ is known to be a trace requirement for algae, the bioaccumulation exhibited was far beyond metabolic requirements. The factors clearly influential in the accumulation of nickel and other metals were pH and contact time.

ALGAL GROWTH RATE AND HYDRAULIC RESIDENCE TIME

In any flow-through system, hydraulic residence time is defined as the volume of the reactor divided by the volume of liquid entering or leaving the system per unit of time. If a volume of liquid greater than that flowing through the system is harvested daily, the cell residence time, related to growth rate, can be made shorter than the hydraulic residence time. On the other hand, if

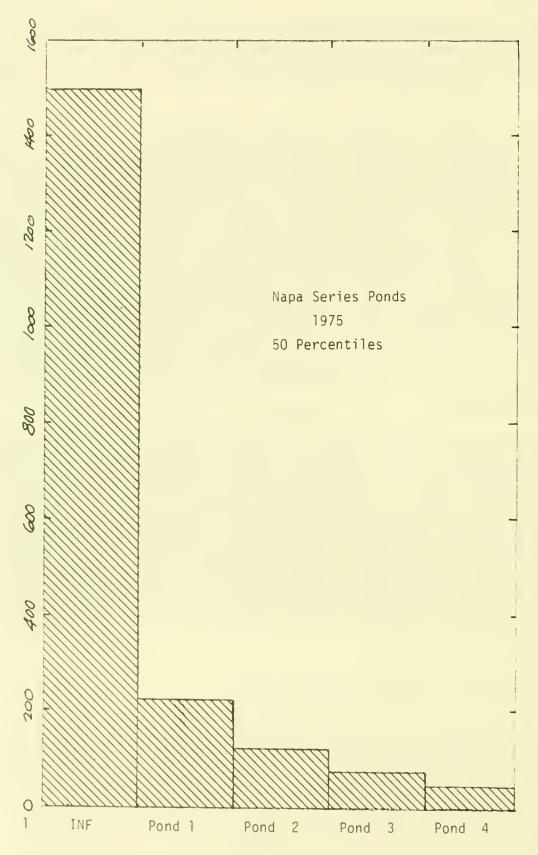


FIGURE 5

TOTAL CHROMIUM REMOVAL IN SERIES PONDS

After Ramani et al (1975)

some harvested cell material is returned to the system, the mean cell residence time may be made greater than the hydraulic residence time. Which of these is optimal for selenium removal is not apparent at this time, and must be determined locally.

In the case of Wang's nickel experiments, cited above, cell age, pH, illumination and species all had interacting effects. Thus, one cannot neglect these factors in studies of algal uptake of objectionable materials for the drainage project.

PHYSICAL CONDITIONS

Among the many physical conditions one must consider in algal uptake studies are depth, light, temperature and mixing. Each of these has a profound effect on algal growth and consequently on selenium and metal adsorption and assimilation. In no case is data available relating these various factors to removal in drainage waters. Conclusions, therefore, can hardly be drawn until studies of the influence of these factors on removals have been made.

PHYSICAL WATER QUALITY CHARACTERISTICS

In studies by Wang (1984), and by the Interagency Group, pH has an outstanding effect on algal growth and ion removal. Similarly, elements such as selenium, with multiple valance states, are deeply effected by both the redox potential or the pH of the system they are in. Uptake data in the absence of these measurements is thus difficult to relate to any hypothetical model. Daily variations in dissolved oxygen is also an influential environmental factor that should not be ignored in a detailed study of heavy metal and selenium removals.

TOTAL DISSOLVED SOLIDS AND MAJOR COMPONENTS IN WATERS

Because of the apparent spacial and temporal variability of drainage waters, it is important to know the influence of total dissolved solids and of the major water quality ions such as sodium, magnesium, calcium, chloride, sulfate and bicarbonate on selenium and heavy metal uptake by algae. For example, it

has been proposed by Jones-Whitthuhn et al (1983) that sulfur concentration influenced the toxicity of selenium to the alga Chlamydomonas reinhardii. "zero" sulfur media, the algal growth rate was reduced 75 percent by 250 micrograms per liter of selenium, whereas with 6 mg per liter of sulfur, selenium concentrations of 750 mg per liter showed no growth inhibition. The possibility that the sulfate ion can also provide such protection was not suggested by Jones-Whitthuhn et al, but in an earlier article, Kumar et al (1971) had shown that sulfate is protective for two species of blue-green algae (Cyanobacteria) Anacystis nidulans and Anabaena variabilis against the toxicity of selenite and selenate (Kumar, 1971) also gave evidence of three-fold difference is susceptibility of the two algae to the selenium compounds, and noted that different growth phases of the algae were also influential. Shrift, (1953) had, much earlier, discussed sulfur-selenium antagonism in the green alga Chlorella vulgaris. Provasoli et al (1974), frequently emphasize the importance of the ratios of the major anions and cations, as well as the importance of bacterial-derived vitamins for algal growth and development in natural systems.

TRACE ELEMENTS

As noted previously, very little data has been collected on the influence of trace elements on microalgae. Most of the information available relates to the toxicity or inhibitory effects of selenite (SeO $_3^-$) and selenate (SeO $_4^-$) on various species of algae. For examples, see TABLE IV. As is apparent in TABLE IV, in this scanty amount of data there are large variations in tolerance for selenium and trace metalic ions: a thirty-fold difference among non-motile green algae species, and a seventy-five-fold difference between motile and non-motile green algae. Even greater differences seem to be manifested in the blue-green algae, ranging from 79 μ /l for Phormidium to 10,000 μ /l for Microcoleus.

The literature leaves no doubt regarding the need for inorganic selenium as an essential nutrient for algae (Shrift, 1953, Lindstrom, 1983, and Gennity et al, 1984), but it usually is not specified in inorganic media (Stein, 1973), probably because it is present in trace amounts as an impurity in many chemicals.

TABLE IV

SENSITIVITY OF SELECTED MICROALGAE TO

SELENIUM, ARSENIC, CADMIUN AND MERCURY

Generic Name	Class	Reported Sensitivity (Micrograms per Liter)			
		Se	As	Cd	Нд
Akistrodesmus (1)	Green	10	100	10	50
Scenedesmus (1)	Green	100	10	10	100
Selenastrum (1)	Green	300	25,000	50	100
Chlamydomonas (2)	Green	750	_	-	-
Microcoleus (1)	Blue- Green	10,000	-	10	400
Phormidium ⁽³⁾	Blue- Green	79	-	-	-
Mixed Green & (4) Blue-greens		300	۰	-	-

⁽¹⁾ After Vocke et al (1980)

⁽²⁾ Jones-Witthuhn et al (1983)

⁽³⁾ Sielick (1972)

⁽⁴⁾ McKeown (1985)

Buttino et al (1984) reported that a marine microalgae <u>Dunaliella primalecta</u> and <u>Porphyridium cruentum</u>, as well as a <u>Chlorella sp.</u>, synthesized selenium amino acids and lipids when grown in artificial sea water containing sublethal concentrations of selenium (said to be 10^{-2} g/l, or 10,000 micrograms per liter). Again, growth conditions were far different than likely to be encountered in high-rate ponds with drainage waters.

One concludes that, although selenium is somewhat toxic to microalgae, there are great variations in the tolerance of various species, and that adaptive tolerance is attainable. It is also apparent that sulfate and perhaps other ions in sea water and brackish waters provide a protective effect, not only against selenium toxicity, but also toxicity of heavy metals. One mechanism by which microorganisms (particularly certain fungi and bacteria) are self-protective is by methylation of selenium to dimethyl selenide, (CH $_3$) $_2$ Se. Shrift (1958). Another protective method by motile green algae is incorporation of selenium in pigments such as carotene (Gennity et al, 1984) and in protein bound seleno-amino acids (Buttino et al, 1984). Clearly, among others, those algae rich in sulfur amino acids (Porphyridium) and in carotenoids, (Dunaliella), should be studied for selenium uptake from drainage waters. Dunaliella in particular grows well in competition with other algae in saline waters.

WATER QUALITY CONDITIONS IN THE KESTERSON AND LOS BANOS AREAS

Extensive analyses have been made by the U.S. Bureau of Reclamation (Anon. 1985) and by Presser et al (1984) for the various trace elements on various sites in the San Luis Drain area. A complete water quality evaluation of the available information in these documents is beyond the scope of this report, but certain characteristics do bear on the objectives of this report, and consequently require some review. In those studies, the analyses of "trace minerals" include: silver, arsenic, boron, cadmium, chromium, copper, iron, mercury, manganese, molybdenum, nickel, lead, selenium and zinc.

Quality Standards for these various ions for protection of fresh water

aquatic life, for irrigation waters and for drinking water, are shown in TABLE V for comparison with the surface waters found at Kesterson Reservoir, the drainage waters at the Los Banos desalting facility of the Department of Water Resources and the drain at the test facility site at Firebaugh, California.

The data in TABLE V indicate that boron at Kesterson, Los Banos and Firebaugh is in great excess of irrigation standards (there are no boron standards for aquatic life or drinking water). Cadmium is in slight excess of drinking standards at all sites, as is chromium. The chromium valence state is not reported. It is possible that the chromium is in the +3 state, and would not be in violation. Copper appears to be within standards at Kesterson, but exceeds standards somewhat at Los Banos and Firebaugh. Iron, lead, manganese and mercury concentrations are within acceptable limits at all sites. Molybdenum greatly exceeds the limits recommended for irrigation water, but there are no established limits for molybdenum in fresh water for aquatic life and for drinking water. Molybdenum is, in fact, known to be a trace nutritional requirement for microalgae, but the required levels are unknown. Nickel appears to be well below the established level, as are silver and zinc at all sites. However, selenium averages about 5 times the recommended 24-hour average concentration and, in some samples, exceeds the standard by 10 fold at Kesterson and by 13 fold at Los Banos. Mean levels for selenium at Firebaugh are about 1/2 their values at the other two sites. For this reason, it is believed that the Los Banos site would provide more representative selenium levels for problem drainage water. Also, the Los Banos site has much more land than the Firebaugh site, which was extremely cramped for space during the 1966 to 1978 drainage investigations.

Accordingly, the Los Banos site is likely to be a more appropriate location for a pilot facility than the Firebaugh site. There are locations where the selenium is much higher than at Los Banos - for example, Broadview Farms on the South side of Nees Avenue, 1 mile east of Brannon, where concentrations of selenium range from 3,400 to 4,700 micrograms per liter, or at Tranquility, West of San Luis Drain and South of American Avenue, where concentrations range from 850 to 1,400 micrograms per liter. These locations, however, would require procurement of land, whereas land is already available at the Los Banos site, and would reduce both expenditures and the amount of time needed to start the studies. Also, any system that significantly reduces selenium levels at Los Banos should work equally well or better where concentrations are higher.

TABLE V

COMPARISON OF TRACE CONSTITUENTS IN DRAINAGE WATERS IN KESTERSON RESERVOIR, THE OWN DESALTING FACILITY

AT LOS BANOS AND THE FIREBAUGH TEST SITE, WITH WATER QUALITY STANDARDS FOR FRESH WATER AQUATIC LIFE, DRINKING WATER AND IRRIGATION WATER

Elenent	Pro (Mic 24 Hour	ater Aquation otection crograms/lin 30 Day Average		Drinking Water Primary Standard µg/L	Irrigation Recommen ور Continuous	ided Max.	Kesterso Surface V yug/1 • Mean • 5		DWR Fa Los B yg Mean	nos /1	Firebaugh µg/L Mean
Aluminum	-	-	-		5,000	20,000	-		-	-	-
Arsento	-	16	140	50	100	2,000	1	2	2.6 + 2	5	1.28 + .48
Boron	-	-	19,000		750	2,000	21,300 ± 12,000	65.000	14.600 -10,000	15,000	7,000 + 4,100
Cadmium	.051		10 * (6.3)*	10	10	50	<1	5	1.12 ± .3	2	<1
Chromium ⁺⁶ Chromium ⁺³	0.29	7.2 130	11 (21) 2,700	50	100	1,000	6.28 * 7	21	18 + 6.6	30	26.4 - 15.8
Copper	5.6	20	29 (43)		200	5,000	3.45 *2.87	15	17.7** ‡ 33	19	9 - 7.6
Iron	-	-	-		5,000	20,000	90.5 -72	300	49 ± 79	90	73.3 + 84
Lead	-	6.4 (20)	160 (400)	50	5,000	10,000	3.57 + 3.5	10	3.5 + 6	16	2 - 1.8
Manganese				,	200	10,000	37 ÷ 25	1 30	76.5** - 67	170	72.8 + 98
Mercury	0.20	0.2	1.1 (4.1)	2	•	•	0.1	12	< .1	.2	0.11
Molybdenum		-	•		10	50	134 - 99	540	133 - 143	480	44.1 ± 8.5
Nickel	160		3,100		200	2,000	20.77 - 22	120	27.7 - 37	110	24.7 21.7
Selenium	35		260	10	20	20	223 ± 1/10	500	315 - 148	4.50	1.22 + 39.5
511ver			13	50	-	•	1	5	<1	< 1	< 1
Zinc	47		570	-	2,000	10,000	27 - 32	160	9.66 - 8.6	20	10.1 - 7.5

Numbers in parenthesis indicate recent changes

Micrograms و س

^{**}Highest values dropped

Another factor which is important is having complete data on major water quality components. A large backlog of data on these components is available from the Los Banos Desalting facility (PRC Inc., 1983). These data are shown in TABLE VI. TABLE VI is accordingly used as a basis for examining the feasibility of using integrated algal bacterial systems as a method of decreasing seleniun, cadmium, chromium, copper and molybdenum in drainage waters.

ALGAE GROWTH POTENTIAL OF DRAINAGE WATERS

Studies by Arthur et al (1969) and by McKeown (1985) indicate that mixed cultures of green algae (and some blue-greens) grow abundantly on drainage waters.

Major requirements for algal growth are: water, carbon, nitrogen, phosphorus, calcium, sulfur, magnesium, molybdenum, iron and manganese, as well as lesser amounts of fifteen other elements. Except for nitrogen, carbon and phosphorus, all other ions are present in abundance. There is, of course, also abundant water from the drain, so hydrogen and oxygen are no problem. According to TABLE VI, bicarbonate ion varies between 391 and 192, with a mean of 214 mg/l. Bicarbonate ion is about 1/5 carbon by weight, so the total amount of carbon from this source varies from 78.2 mg/l to 38/4 mg/l, with a mean of 42.8 mg.l. In addition to inorganic carbon (PRC ENG, 1983), indicate that the drainage waters contain 1 to 14 mg/l of organic carbon. Because any ponding system to be used will integrate waters over a number of days, variations are likely to be minimized so that mean values for the various nutrients are likely to be more representative than the extremes. Thus we shall assume a mean organic carbon of 4.6 mg/l and an inorganic carbon of 43 mg/l for a total of 47.6 mg/l of carbon.

In TABLE VI, nitrate is given as NO_3 , which is 22.6 % nitrogen. Levels for nitrate in the drainage water are reported in this TABLE as 234, 3.2 and 97 mg/l. Again, using the mean of 97, the projected amount of nitrogen is 97 X 0.226 = 21.9 mg/l as N.

Phosphates are given as P in TABLE VI, so no transformation is required. The

TABLE VI

RANGE OF INDIVIDUAL CONSTITUENTS REPORTED IN SAN JOAQUIN VALLEY AGRICULTURE DRAINAGE WATERS

Parameters	Conc	entration(a)
	Maximum	Minimum	Average(b)
Calcium	590	55	369
Magnesium	583	54	161
Sodium	5,250	400	1,093
Potassium	7.0	0.9	4.0
Bicarbonate Sulfate Nitrate (NO ₃) Chloride	391	132	214
	10,100	607	2,282
	234	3.2	97
	2,680	110	987
Manganese	0.36	0.04	-(c)
Iron	12	0.04	2,1
Strontium	4.8	3.6	-(c)
Boron	34	1.4	12
Silica	60	7.2	26
Phosphates (as P)	0.15	0.01	0.04
Total Organic Carbon	14	1.0	4.6
	8.0	7.0	7.3
	21	7	16
	715	1	54
	20,700	1,930	5,342
Electrical Conductivity (µmho/cm)	21,500	2,250	6,462

⁽a) Concentrations of chemical constituents are expressed as milligrams per liter (mg/l) of constituent unless stated otherwise.

(c) Only two samples.

From PRC Eng. Inc. (1983)

⁽b) Average based on 5 to 200+ individual samples

mean amount of phosphate is 0.04 mg/l

A rule of thumb is that microalgal biomass potential of a water is 2.5 carbon, 12 X nitrogen and 100 X phosphorus. According to this formula, we have TABLE VII, which shows that, to attain a concentration of microalgae of 300 mg/l, it will be necessary to add a minimum of 77 mg/l of carbon, 3.16 mg/l of nitrogen and 2.6 mg/l of phosphorus.

Values in TABLE VII are for the mean C, N and P in the drain, which may have considerably different concentrations at Los Banos. Obviously, in any on-going system, nutrient adjustments would be made on a day-by-day basis.

THE PILOT SYSTEM

Under the previous topic "Characteristics of Advanced High-Rate Ponds", much of the theory of such ponds was advanced. In this section the design of this particular system is discussed.

Two approaches to our design can be taken.

- 1. Select a flow and design a system to accomodate it.
- 2. Select a high-rate pond size and design the remainder of the system and the flow to be handled on the basis of that size.

Although any ultimate system would need to be handled by the former approach, we feel that the second approach is more practical for a pilot demonstration system, since it will better control capital input. We have, therefore, arbitrarily selected four 1-acre high-rates ponds - two to be fully lined with membranes and two to be earth-lined at the bottom but lined along the levees for weed control.

Justification for the one-acre size is that it is sufficiently large to serve as a prototype and yet makes economical use of research funds. We assume land is available at the Los Banos site, and that no land costs will be involved. We further assume that the soils are suitable for grading and compaction without special treatment other than moisture control.

TABLE VII

ALGAL BIOMASS POTENTIAL OF UNFERTILIZED DRAINAGE WATER SHOWING FERTILIZERS TO BE ADDED TO MAKE A BALANCED WATER FOR 300 MG LITER OF MICROALGAE DRY WEIGHT BASIS

Element	Concentration in Drain mg/l	Multiplier	AGP mg/l	Deficit mg/l	To Be added mg/l
С	42.8	2.5	107	193	77.2
N	21.9	12.0	262	38	3.16
Р	.04	100.0	40	260	2.60

System Concept

A schematic diagram of the proposed system is shown in FIGURE 6. Rationale for the system follows:

High-Rate Pond

It is established that microalgae take up some selenium and have an affinity for heavy metals. To maximize this uptake, the high-rate ponds will be designed to utilize not only all available nitrogen, but to maintain an algae concentration of 300 Mg/l.

Subsidence Chamber:

Arthur (1969) has shown that algae and bacteria grown symbiotically on drainage water have a high tendency to settle. We will provide a subsidence chamber to permit such sedimentation. This chamber will feed into a fermentation pit, where concentrated algal bacterial biomass can ferment under anoxic conditions, known to produce organic acids.

Carbonation Chamber:

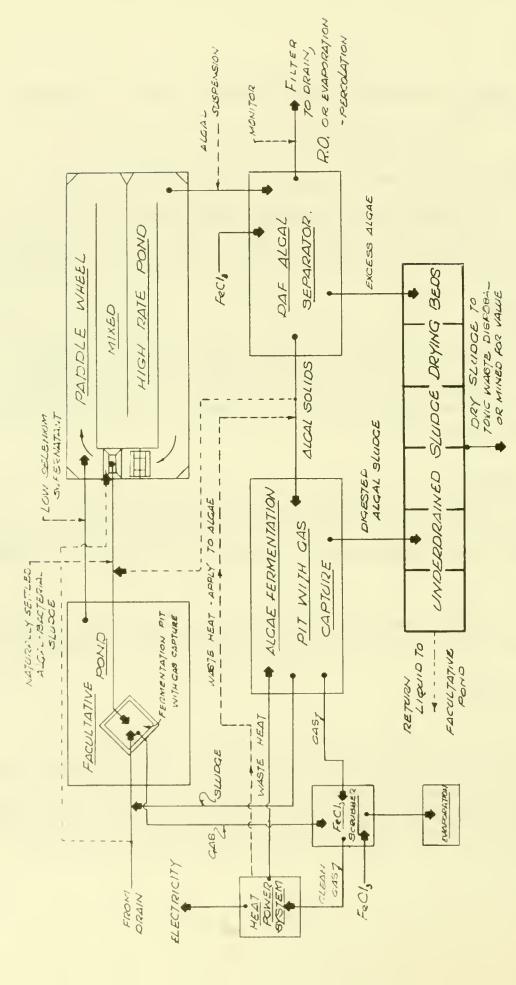
It is clear from TABLE VII that carbon dioxide will need to be added to maximize nitrogen uptake and to control pH in the pond. Carbon dioxide addition will be counter-current carbonation in the channel by means of a special chamber.

Paddle Wheels:

Paddle wheels of the type shown in FIGURE 2 are economical and simple to install; they efficiently provide a variety of mixing velocities for an experimental system.

Earthwork Berms and Levees:

These are economical and durable when lined. In ponds without bottom lining, the berms will be lined, one set with heavy plastic and one with gunnite. Both types of embankment protection have good and bad points. For example, gunnite is costly and often cracks, but does not deteriorate, whereas heavy plastic does not crack and is less expensive, but eventually deteriorates in sunlight and must be replaced.



PONDING SYSTEM FOR HELVY METAL AND CELENIUM REMOVAL FROM SUBCLIFACE TILE LEAINAGE WATER SCHEMATIC DIAGRAM OF CANSIDATE INTEGRATED FICURE 6

Linings:

One pond with bottom linings will be lined with asphaltic plant mix and one with 0.030-thick chlorinated polyethylene. Of the two earth-lined ponds, one will have local earth compacted and one will have an imported clay liner. Both will have facilities to determine whether or not their contents contribute to ground water contamination.

Headworks and Subsidence Chambers:

The headworks and subsidence chambers will be monolithic concrete, to maintain dimensional accuracy and to provide a stable working area.

Facultative Pond:

The facultative pond shown in FIGURE 6 will be designed so that the bottom pit is extremely anoxic, and it will be provided with a gas capture system. It is expected that emergent gases will contain methane and both hydrogen sulfide and hydrogen selenide. Since the latter two are highly toxic and since the former is valuable, all gases should be captured. The gases will accordingly be captured and scrubbed with FeCl $_3$ water to remove $^{\rm H}_2{}^{\rm S}$ and $^{\rm H}_2{}^{\rm Se}$, leaving $^{\rm CH}_4$ to be burned to produce electricity.

DAF Unit:

Experience with microalgal systems indicates that autoflocculated algae or algae treated to flocculate are quickly and efficiently removed with dissolved air flotation (DAF). A DAF system is provided to assure that all algae are removed. FeCl₃ will used as a scavenger for any reduced selenium present. The DAF unit water can be filtered through a rapid sand filter if the water is to enter a reverse osmosis system for reclamation. If the water is to go to an evaporation-percolation system, filtration will not be needed. Combined DAF and filtration may be accomplished in a Krofta (1983) sandfloat TM system.

Algae Fermentation Pit:

There are several reasons for this part of the system. The quantities of algae that will be produced in full-scale systems will be prodigious, and it will be worthwhile to recover their energy in the form of methane. Thus, experience with low-cost methane fermentation of algae in ponds with gas capture is an important part of this study. Also, since substantial amounts of supplemental

carbon dioxide are needed to reach 300 mg/l of algae in the HRP, the methane should be burned in a heat power generator to produce CO_2 , heat and electricity. The electricity produced will be far more than enough to turn the paddle wheels and to operate the systems' pumps. The waste heat applied to the algae entering the fermentation pit will accelerate algal fermentation and the CO_2 will be used in the high-rate pond to provide carbon and to control pH.

Gas Scrubbers:

Because the ${\rm CH_4}$ for the heat power generator should be freed of ${\rm H_2S}$ and ${\rm H_2Se}$ and other corrosive gases, a scrubbing system will be required. As noted above, this unit will be used to scrub both the gases from the facultative pond pit and from the algae fermentation pit. An ${\rm FeCl_3}$ solution is likely to be the most economical and effective chemical for gas scrubbing, since it should scavange any ${\rm H_2Se}$, converting them to insoluble iron sulfides and selenides. As noted previously, it is important to scrub these gases anyway, because they are toxic and should not be returned to the air.

Scrubber Water Evaporation Pit:

Scrubber water is likely to be very toxic and should not be permitted to enter surface or ground waters. Accordingly, a lined evaporation pit is provided.

Underdrained Sludge Drying Beds:

Surplus algae from the separator and algal sludge from the algae fermentation pit will be dried in sand beds. It will be necessary, however, to capture the underdrain drainage waters and to return these to the facultative pond. It is possible that surplus algae can be used for a fish or animal feed supplement, but toxicity studies should be carried out because they may have adsorbed an excessive amount of heavy metals or other toxic elements.

Specific Element Design:

The detailed design of specific elements of the pilot plant system, shown in FIGURE 6, is beyond the scope of this report. However, it is necessary

to size the elements in order to have a basis for preliminary cost estimates.

High-rate Pond Design:

Depth:

To attain 300 mg/l of algae, use equation (4C) $d = 9,000/C_{\text{C}} = 9,000/300 = 30 \text{ cm}$ Use depth of 12"

Wall Heights:

To prevent splash-out, use 2'6" levees, but channel dividers can be 2'0"

Channel Width:

For a 20' paddle wheel, ideal channel width is 40'. Use 40' at waterline

Channel Length:

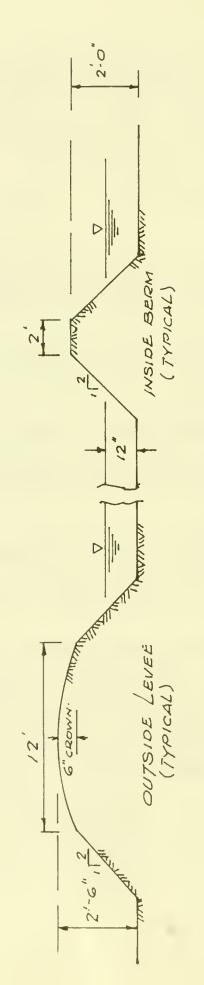
Since ponds are to be one acre and channel width is 40', the length of channel must be $43,500 \text{ ft}^2\text{ac}^{-1}/40' = 1,089'$, but because there are contraction sections at 20' paddle wheel, use a channel length of 1,200'.

Pond Length:

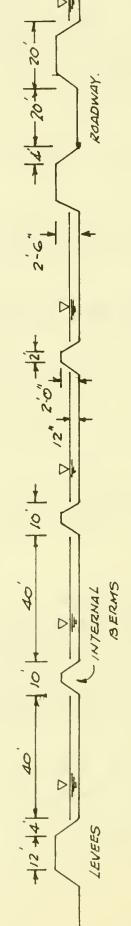
Since there are 2 channels per pond, overall pond length at the waterline is 1,200/2 = 600 ft., but due to levees, overall length will be 636 ft.

Outside Levees:

Will be 2 feet high, but will have a 6-inch crown. These levees add 20 feet to each end and sides, therefore the overall length, including berms, would be 640 feet. Allowing 2-foot overlap at waterline on each end subtracts 2 feet, so overall length is 636 feet. Similarly, widths of channels at waterline are to be 40 feet, so internal berms occupy 5 feet, which, with two 12-foot levees and four 40-foot channels, amounts to 214 feet. The area to be occupied by the four ponds, allowing for a 20-foot roadway all around and between each pair, will be 676 feet X 488 feet, or about 7.5 acres. A cross section of the ponds is shown in FIGURE 7, and a layout is shown in FIGURE 8.



LEVEE AND BERM DETAILS (NTS)

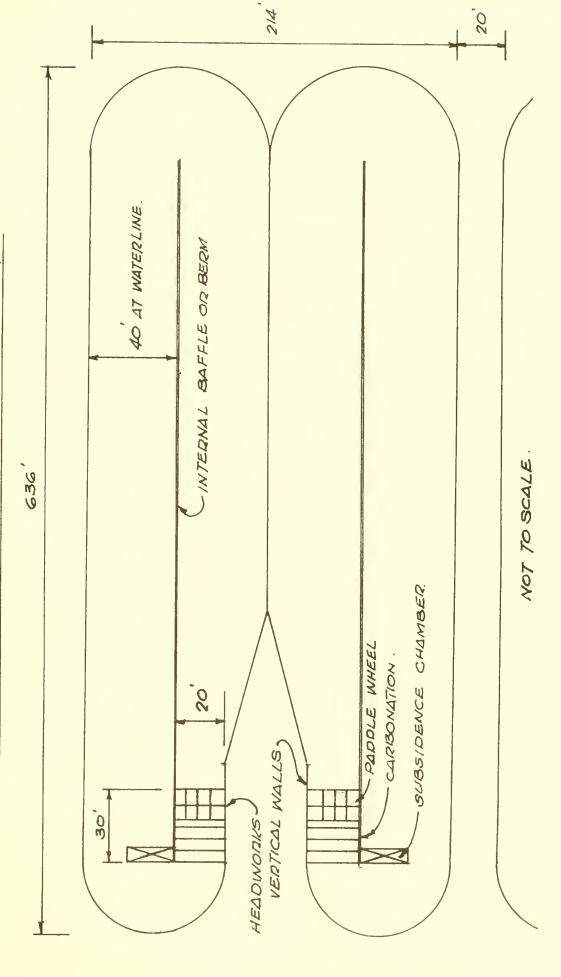


ONE OF TWO PAIRS OF PONDS

CROSS SECTION OF HIGH RATE PONDS LEVEE AND BERM DETAILS.

- 54

TREATMENT OF ACRICULTURAL DRAIN WATER.



FICURE 8 PLAN OF ONE OF TWO PAIRS OF HICH RATE PONDS.

Flow Rates:

In order to design the remainder of the systems shown in FIGURE 6, it is necessary to calculate flow rates through the system. The highest flow rates will, of course, determine the design. The highest flow rate is calculated below.

Pond Volume and Residence Time:

Each 1-acre high-rate pond contains 1 acre-foot, or 326,000 gallons of water. Minimum residence time is likely to occur at a time when solar energy is at its' maximum. At that time, net photosynthetic efficiency is likely to be only about 2.5 percent, so: assume a solar input of 600 cal/cm²/day, and the pond depth is 30 cm. Using the author's formula (Oswald, 1960) for residence time:

 θ = h Cc d/1,000 FS., where θ is the residence time, and substituting the heat of combustion of algae (h) 5.5 cal/mg, depth (d) is 30 cm and solar energy (S) is 600 cal/cm²/day and F is 0.025; $\theta = 5.5 \times 300 \times 30/1,000 \times 10^{-2}$.025 X 600; $\theta = 3.3$ days.

Also $\theta = V/Q = V/\theta - 326,000/3.3 = 98,787$ gallons per 1-acre pond per day. Therefore the highest flow rate is assumed to be about 100,000 gallons per day.

Facultative Pond Design:

The facultative pond design involves two major components: an internal digester and an outer pond.

Internal Digester:

On the basis of past experience with such units, these are designed to be 10 feet deep and to have a hydraulic residence time of 2 days. We will assume one facultative pond serves two high-rate ponds. So Q = 200,000 gallons per day or, for two days 400,000 gallons at 7.48 gallons/ft 3 = 53,476 ft 3 . At 10 ft depth, the area is 5,347 ft 2 , or about 73 feet on a side. Accordingly, the internal digester for the facultative pond will be 73 ft. X 73 ft. X 10 ft. deep. It will serve two high-rate ponds.

Outer Pond:

The remainder of the facultative pond should also be 10 feet deep and have a residence time of 10 days. $200,000 \times 10 = 2 \times 10^6$ gallons, or 265,379ft. 3 = V at 10 foot depth and area = 26,700 ft 2 , or about 164 feet on a side. A sketch of the facultative pond profile is shown in FIGURE 9A.

DAF Design:

Experience has shown that DAF units only require a residence time of about 30 minutes or less. We will have one DAF unit for two high-rate ponds. Thus, A = 200,000 gallons per day = 200,000/1,440 min/day = 138 gallons per minute. For 30 minutes = 4,166 gallons. Use a 5,000 gallon DAF tank for each set of ponds. A sketch of the DAF unit is shown in FIGURE 10. A Krofta 1983 DAF unit is smaller than that shown in FIGURE 10, since the required residence time is likely to be less than 10 minutes. Accordingly, if economical, Krofta units may be substituted for those shown in FIGURE 10.

Algae Fermentation Pit:

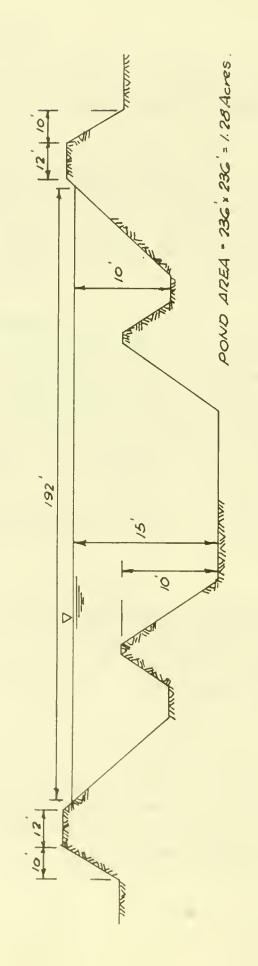
The maximum rate of algae production will be 100,000 gallons X 3.78 liters/gal X 300 gm/l = 1.134 X 10^8 mg/day/ 10^6 mg/kg = 1.134 X 10^2 = 113.4 kg/day. There will be one fermentation pit used for each pair of high-rate ponds. Therefore, the fermentation pits should be able to handle and ferment 226.8 kg/day or 226.8 X 2.2 = 589 lbs per day. Permissible loading on plug flow pond digesters at 70^0 C with 2 percent influent solids is .026 lbs/ft³, and so the digester should have a volume of 589/.026 = 22,653 ft³, say 22,500 ft³, if the pit is 15 feet deep, the area is 1,500 ft². If the pit is 20 feet wide, its' normal length will be 75 feet. A profile of the pit, including the surrounding levees, is shown in FIGURE 9B.

Sludge Bed Design:

The underdrained sand beds should be 5 percent of the area of the high-rate ponds. Since the ponds are 4 acres, the algae drying beds should be 4 \times .05 = 0.2 acres, or 8,712 ft². If the beds are 40 feet wide, they should be 220 feet long. There will, then, be 8,800 ft² of sludge beds.

Gas Scrubber:

The rate of gas production should be about 8,000 ft 3 /day, or about 6 ft 3 per minute. A 10-minute residence time for gas is required with gas recirculation and equal volumes of gas and liquid, the scrubber tank volume is 120 ft 3 . Assume a height of 10 feet, the circular tank area is then 12 ft 2 TT D 2 /4 = 12 D = $\sqrt{48/TT}$ = 4 ft. Thus, need a 4-ft.diameter by 10-ft. tank.



A. FACULTATIVE POND PROFILE

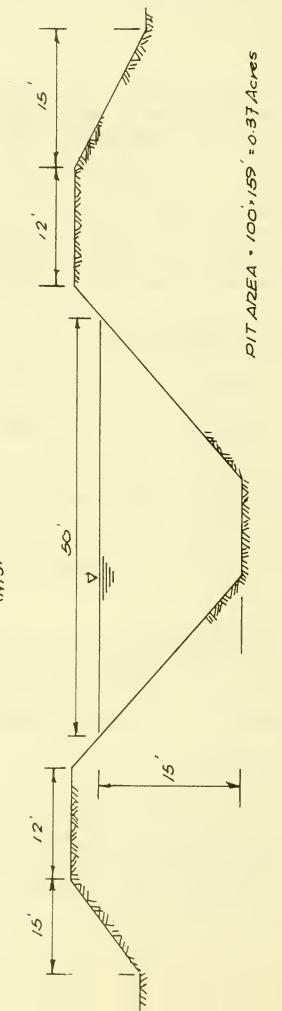
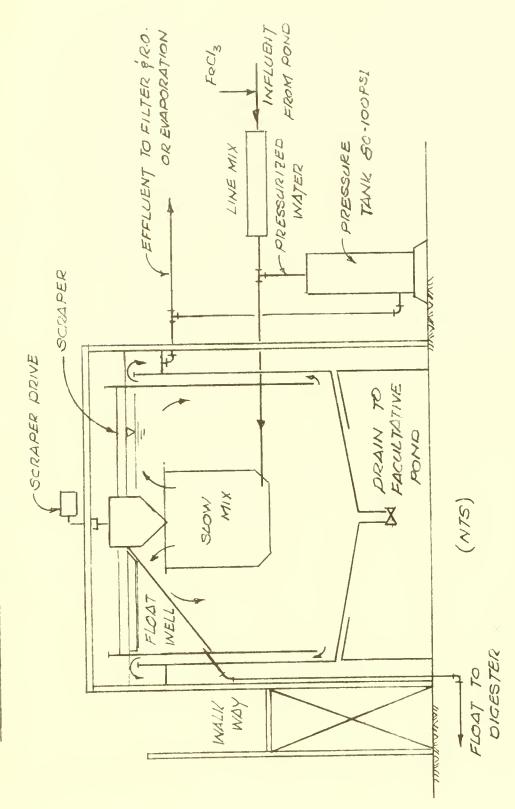


FIGURE 9 PROFILES OF FACULTATIVE AND DIGESTION PONDS B. FERMENTATION PIT PROFILE (NTS)

TREATMENT OF LIRICULTURAL DRAIN WATER



DISSOLVED ANS FLOTATION UNIT FOR ALGAE CONCENTRATION FIGUNE 10

Heat Power Unit:

Each kilogram of algae has an energy content of about 22,000 BTU; at 50 percent conversion in the digester, 11,000 BTU per kilogram are produced so at 1 Kw hr per 10,000 BTU, there are 1.1 Kw hr/kg. As two ponds produce a maximum of 226.8 kg of algae per day, the maximum energy output is 226 X 1.1 = 248.6 Kw hrs/acre/day. Thus, about 114 Kw hrs of net energy will be available. One generator will be used for each pair of ponds. Thus, the generator should have a capacity of 248/24 = 10.33 Kw. Use a 15-HP output, 50 HP input motor generator system for each two ponds.

OPERATIONS

High-rate Pond

If it is properly designed and constructed, the operation of a high-rate pond is quite simple. The steps are:

- 1. Add water and nutrients and start paddle wheels. Adjust rotation so velocity of water in the channel is about 0.5 feet per second.
- 2. Inoculate the pond with algae or permit indigenous algae to develop.
- 3. When pond is green, begin to check pH. When pH rises to 9, begin to add bicarbonate and CO_2 to maintain pH near 8.2.
- 4. Add phosphate as ammonium phosphate and EDTA + iron as required. When concentration of algae reaches 300 mg/liter, begin harvesting and data collection. Initially, most of the harvested algae should be put in the facultative pond to create an intensely anoxic zone through which all drainage water would eventually pass. As algae develop their expected subsidence characteristics, a larger and larger fraction of the algae can be harvested for energy production. Occasionally, during the first months of operation, the pond may lose most of its algae to predators but, as time goes by, this problem should diminish as resistant strains of algae develop. Rotenone or other pesticides for algal predator control can be used as a last resort.

Facultative Pond

This unit should be designed to be self-operating. As it fills, it, too, may

go through periods of predation, but these will dampen with time.

DAF Unit

Extensive experience now exists with the operations of DAF units. It will be necessary to use jar tests and effluent monitoring to assure complete algae removal. FeCl $_3$ is used as the coagulant because, according to Presser et al (1984), the ferric ion will form insoluble precipitates with selenite and (Fe 2 (OH) $_4$ SeO $_3$) is formed. The point of addition of FeCl $_3$ and polymer, if needed, also must be determined in situ.

Algae Fermentation Pit

A considerable amount of experience now exists in the operation of plug flow digesters for cattle manure. The fermentation of algae is similar and has been studied extensively (Golueke et al, 1957) (Eisenberg, 1979), and no unusual problems are expected. It will be beneficial if waste heat from the generator can be applied to the algal sludge prior to its entry into the digester. This heat will kill the algae and improve the digestability (Chen, 1985). Concentrated algae from this pit will be applied to the underdrained sand beds, although some may be required to aid in maintaining anoxic conditions in the facultative pond fermentation volume. The gas recovery systems in both the facultative pond and the algae fermentation pit are under patent consideration by the University, and details of these elements will be revealed after a formal patent application is complete and filed. Plastic covers will be used on the pilot system if delays on the patent applications are encountered. Such covers have been used successfully on farm ponds in many locations.

Sand Beds

Operation of algae drying beds has been established for many years. The crucial factor is not to draw more than a few inches of sludge, so that it dewaters and drys quickly. The underdrain sump should be promptly pumped into the facultative pond to avoid algae growth in the liquid and its reaeration to form SeO_A .

Gas Stripping and Heat Power System

The only unusual factor in gas stripping is the use of ferric chloride. It may be that other metallic ions such as magnesium chloride would be an

improvement, but local studies are required to make this decision. Because of its small size, the heat power unit should be a gas-diesel driving a conventional A.C. generator.

STUDY PLAN FOR PILOT SYSTEM

Preliminary Laboratory Studies:

Although, as indicated above, much of the required information for design of a system to study removal of selenium and other objectionable trace elements is available from the author's prior laboratory and field studies and experience with similar systems, some critical factors are unknown and therefore will require preliminary laboratory studies prior to finalizing the pilot plant design.

The major unknowns requiring immediate study are:

- 1. The influence of selenate and selenite at concentrations of 2 to 5 mg/liter on methane fermentation of microalgae.
- 2. The rates of conversion of soluble selenate to insoluble selenite in anoxic algae fermenters, when influent selenate concentrations are 2-5 mg/l, and at various temperatures.
- 3. The rate of precipitation of anoxic selenite-algae mixtures with inorganic polyelectrolytes such as FeCl_3 , AlCl_3 and $\text{Al}_2(\text{SO}_4)_3$, and by various organic polymers.
- 4. The influence of selenium concentrations on the growth of algae which appear and grow readily in continuously mixed, enriched and illuminated shallow ponds containing drainage waters.
- 5. The degree of uptake of selenate from drainage waters by continuously mixed, enriched and illuminated microalgae cultures indigenous to the drainage waters.
- 6. The degree of removal of selenate from drainage waters by continuous cultures of <u>Spirulina</u>, <u>Dunaliella</u> and <u>Porphyridium</u>.
- 7. The rate of precipitation of hydrogen selenide gas by ${\rm FeCl}_3$, ${\rm Fe}_2({\rm SO}_4)_3$ solutions and perhaps other electrolytes.

These studies, once initiated, would require various amounts of time and may be conducted in several locations. For example, from the above list, Numbers 1, 2 and 3 can best be carried out quickly where algae methane fermentation studies are already in progress. Items 4, 5 and 6 should be studied where

drainage waters of the quality to be used in the pilot plant are readily available. They should be done in paddle wheel mixed mini ponds, in situ out of doors and should continue for several months.

Item 7 should be determined in a few days in any well-equipped chemical laboratory.

Obviously, as a study of this magnitude develops, other laboratory studies will be needed. However, the information from Items 1, 2, 3, and 7 should receive highest priority, since they will be needed to perfect the pilot plant design outlined above. Items 4, 5 and 6 are mainly to develop cultures to be used in the pilot system and can progress as the pilot system is under design and construction.

Pilot Plant Operation:

The 4-1-acre pilot units, once constructed, must be operated on drainage water to determine optimum depth and residence times, to measure algal and energy productivity and to measure removal of selenium and heavy metals.

Environmental factors which must be observed are solar energy, air and water temperature, rainfall, evaporation, relative humidity and wind velocity. Internal pond parameters are pH, temperature, conductivity, algae concentration and suspended solids removals attained. Algal productivity and selenium and metals removal should be measured. The variables such as depth, residence time and mixing velocity can be dealt with in matrix-type experiments, in which one pond is a control and the others are operated according to a preplanned experimental design.

Carrying out such experiments properly at the selected site will require a full-time operator plus an expert laboratory analyst, a well-equipped laboratory and a project engineer. Computer storage of project data will be beneficial for retrieval and processing matrix data, and preparing the final report and cost estimates.

Although an estimate of the cost of the proposed laboratory and pilot plant study is beyond the scope of this report, some idea of the cost magnitude is pertinent. Experience has shown that small (l-acre) lined high-rate ponds

cost on the order of \$100,000 per acre, and small, unlined high-rate and facultative ponds cost on the order of \$25,000 per acre. DAF units of the required size may cost \$125,000 each, if purchased, and somewhat less if constructed of temporary materials on site. From this, it should be apparent that a well-designed and constructed 4-acre pilot system of the type outlined, may cost between one and two million dollars for construction alone, and several hundred thousand per year for proper operations, maintenance and data collection. Because of the great difference in cost between lined and unlined ponds, a major output of the pilot system could be a proof that high-rate ponds become "bio-lined" and do not contribute their contents to the adjacent groundwaters when under continuous operation.

Although we have not, in this report, estimated the exact cost of the proposed pilot system, it is germain to the overall drainage investigation to have a preliminary estimate of the cost of selenium removal treatment in a full-scale algal/bacterial system of the type proposed. For this reason, in the following sections, we attempt to accurately determine the likely current cost of a one-million-gallon-per-day system and from that, using well-established cost relationships for various sizes of sewage treatment systems, to extrapolate the cost of 10 MGD, 100 MGD and 200 MGD systems.

COST ESTIMATE FOR 1 - MGD SYSTEM

A major assumption in the following cost analysis is that the proposed biological system in FIGURE 6 or a modification of it, does decrease selenium in the process stream to a level which is satisfactory under current standards.

Size: We will assume a unit that is capable of being mixed with one 20-foot paddle wheel; about 10 acres, capable of processing 1 MGD. We will further assume that, inasmuch as algae "clog" filters, the biological lining that they form will be satisfactory and that only the levees and berms require lining to prevent weed growth. The channels will be 10,750 feet long. Each side of the channel requires a 10-foot strip of PVC. Allowing for burial of the edges, the area will be 10,750 X 10 X 2, or

215,000 ft². The cost will be 90c per foot² in place, or \$.90 X 215,000 = \$193,000.

Outside berms 3 feet high and 12 feet wide at the top involve 3,233 feet @ 2 yards per foot = $6,466 \text{ yards}^3$ of earthwork. Channel dividing berms will be $(10,750 \text{ X 2}) - 3.233 = 21,500 \text{ ft} - 3,233 = 18,267 \text{ L.F.} @ 0.88 \text{ yards}^3$ per foot = $16,237 \text{ yards}^3$.

Continuing with earthwork, the facultative pond will have a volume of 10 million gallons and, at 10 feet deep, will occupy 133,689 $\rm ft^2$, and will be about 366 ft on a side. The outside levees have a volume of 4.1 yards $\rm ^3/ft.$, so the volume of earthwork is 4.1 X 366 X 4 = 5,964 yards $\rm ^3$.

The internal pit will have a volume of 2 million gallons or $267,379 \text{ ft}^3$ at 10 feet deep. The area is $26,738 \text{ ft}^2$ or 163 feet on a side. The berm length is 654 feet, and will require $3.33 \text{ yards}^3 \text{ ft}^{-1}$. Total yards = $2,180 \text{ yards}^3$. This is also approximately equal to the cut.

Fermentation Pit:

For 1 MGD, maximum algae production is 1,134 kg/day or 2,495 lbs/day. The digestion pit volume is 2,495/.026 lbs/ft 3 /day = 95,953 ft 3 at 15 feet depth. The area is 6,396 ft 2 . Use a width of 60 feet and the length is then 107 feet. The perimeter is (88 + 135)x = 446 feet. Volume of levee is 6.75 yards 3 /ft. = 3,006 yards 3 . Add 1,000 yards for miscellaneous excavation, and earthwork total is 37,859 yards 3 @ \$3.00 per yard = \$114,000.

Liners:

Both the algae fermentation pit and the fermentation pit in the facultative pond must be lined with 30 mil C.P.E. or equivalent. The facultative pond fermentation pit will require 20,000 ft 2 of C.P.E. @ 90¢ per foot, or \$27,000. The algae fermentation pit is 20,000 ft 2 at 90¢ per ft 2 = \$18,000.

Costs are summarized as follows:

Weed Deterent Lining	\$193,000.00
Grubbing and Organic Material Removal	6,500.00
Earthwork	115,000.00
Gas Capture Systems @ \$1.50/ft ²	75,000.00
Liners	45,000.00
Paddle Wheel in Place	30,000.00
Sludge Drying Bed - 20,000 ft ² @ \$1.00/ft ²	20,000.00
1-MGD DAF Unit	60,000.00
Gas Scrubber	15,000.00
Pipes, Motors and Controls	50,000.00
Fencing, Etc.	20,000.00
Access Improvements	25,000.00
Power Generator	75,000.00
Electrical	60,000.00
Contingency @ 10%	80,000.00
Engineering @ 13.5%	117,000.00
Inspection @ 7%	61,000.00
Insurance @ 2%	17,000.00
	43 064 500 00
TOTAL:	\$1,064,500.00

Interest and Amortization, 25 years @ 8%
\$99,700.00 per year = \$273.00 / MG

OPERATING EXPENSES

2 Operators at \$25,000/year	50,000.00
Chemical, FeCl, 146 tons @ \$120.00/ton	17,520.00
Mobile Equipment (estimate)	25,000.00
Carbon Dioxide, 870,000 lbs @ 4¢/lb	26,100.00
Nitrogen Fertilizer, 9,260 lbs @ 6¢.1b	600.00
Phosphorus Fertilizer, 7,900 lbs @ 10¢/lb	790.00
Gasoline for Vehicles	1,200.00
Misc. Herbicides	500.00
Repairs	15,000.00
Administration (prorated)	15,000.00
Total Operational Expense / year	\$151,710.00

Operative expense per million gallons - 151,710.00/365 = \$416.00/MGInterest Amortization - 99,700.00/365 = \$273.00/MGOverall costs are then estimated to be \$416.00 + \$273.00 = \$689.00, or say about \$700.00 per million gallons in a 1 MGD plant.(\$228 acre-foot -1)

VALUE OF ENERGY

At 1,000,000 gallons per day and 300 mg/l algae production is $2.78 \times 10^6 \times 3 \times 10^2 = 11.34 \times 10^8$ mg/day = 1,134 kg/day. At 1 Kw hr per kg of algae, we get 1,134 Kw hrs/day, of which 100 Kw hrs are used for mixing the ponds and pumping fluids. A balance of 1,034 Kw hrs per day, or 377,000 Kw Hrs/year @ 0.084/Kw Hr, is worth about #30,000, or about \$83.00 per million gallons. This would reduce operating expenses to \$333.00 per MG.

One of the major cost items is carbon dioxide. If power is generated, most or all of the carbon dioxide could come from this source. This would save about \$20,000 per year, or \$55.00 per million gallons.

Recent studies indicate that autoflocculated algae can be removed by 60 psi DAF. If most of the $FeCl_3$ could be eliminated, a savings could amount to \$5.00 per MG, bringing the total cost to near \$540.00/million gallons in a 1-MGD facility. (\$176 acre-foot⁻¹).

DISCUSSION

It is clear that a 1-MGD plant is not very economical, nor is it likely that even with credit for energy generated and savings on CO_2 and FeCl_3 , it could become economical. The problem is esentially one of scale. Judging from well-known experience with sewage treatment plant costs, 10-MGD or 100-MGD plants would be much more economical than a 1-MGD plant, since personnel and equipment could be utilized more efficiently and the volume of reactor obtained per unit of cost increases rapidly. For example, if cost decreased with plant size at the same rate as the trends for sewage treatment plants, unit costs for a 10-MGD plant would be about 1/3 of unit costs for a 1-MGD plant and

costs for a 100-MGD plant would be about 1/5 of unit costs for a 1-MGD plant. If such trends were applicable to the proposed selenium removal systems, all costs for treatment in a 10-MGD plant would be \$225.00/MG, \$73.00/AF and, in a 100-MGD plant, would be \$140.00/MG. Since the value of energy generated per unit of value should remain constant, near \$83.00/MG, a 200-MGD plant should nearly break even, and a 500-MGD plant should actually yield a small net profit. Surplus energy production from a 100-MGD facility should be near 40,000,000 Kw hrs per year. This could have a significant impact in decreasing energy imports from Northern California.

Environmental Impact

Concerns are often expressed on the likelyhood that treatment ponds in the valley will be attractive to wild fowl and that a repetition of the Kesterson problems will be promulgated if such ponds are used.

It should be noted in response to these concerns, that high-rate ponds, being narrow, are difficult wild fowl landing sites, which can be made more difficult by cross wires or deterred by close-in hazing guns. It also should be noted that residence times in the high-rate ponds are likely to be only a few days, so that little evaporative concentration of toxic elements occurs there. Also, if the reduction pond scheme proves workable, there will be very little selenium or trace metals remaining in the impounded waters having open, accessible surfaces. These are concepts that can only be proved or disproved in large-scale systems of the type proposed in this study.

CONCLUSIONS

- 1. It is impossible to project the degree of removal of selenium that can be attained in properly designed high-rate ponds. Accordingly, it is necessary to perform in situ laboratory and pilot plant experiments to determine removal effectiveness.
- 2. Operation of a small, in situ facility, employing 4 one-acre

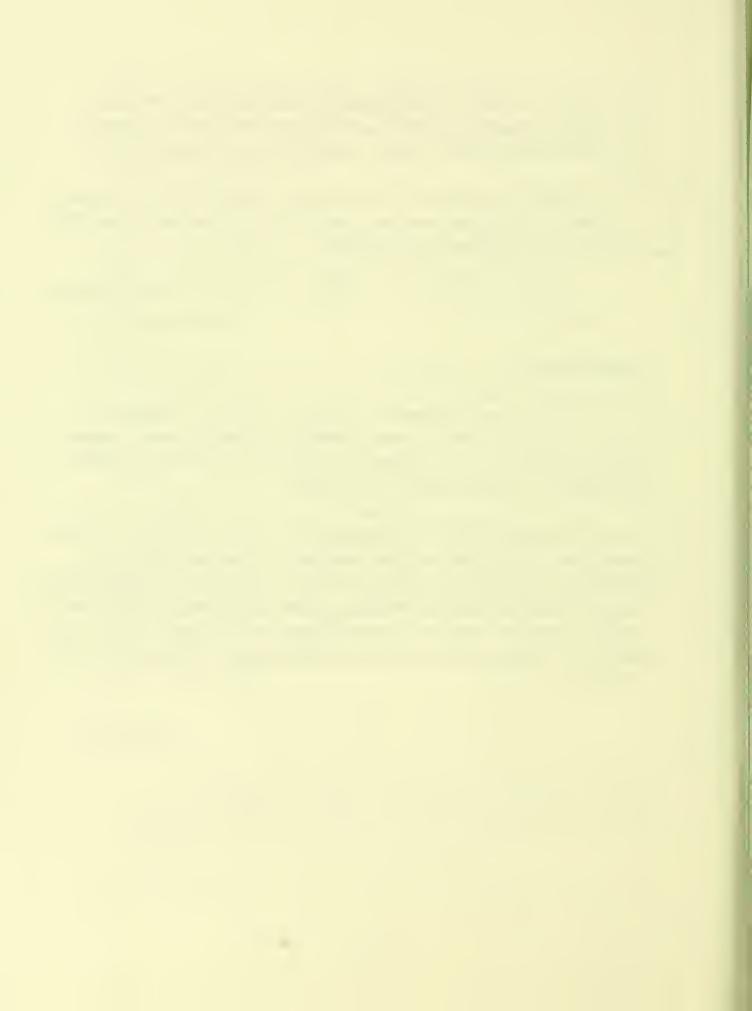
high-rate ponds and supporting plant separation and fermentation systems for one or two years, will be required before the technical and economical feasibility of algal systems can be determined.

- 3. Large algal-bacterial systems have a high probability of selenium removal, but again, experiments are lacking to prove their capability or to accurately project economics.
- 4. The facility should be located near or at the existing Los Banos facility.

ACKNOWLEDGEMENT

I am indebted to Loessa Swanson for typing this manuscript under very difficult conditions and to Lorne E. Swanson and James M. Moore, Swanson-Oswald Associates, Inc. of Lafayette, California for reviewing the manuscript and for preparing some of the figures.

I am also indebted to Mr. Brian McKeown, Mr. Benito J. Marinos, Mr. Phillip Daniel, Ms. Laurie Hinds and Yakup Nerdogan, graduate students at the University of California, Berkeley, for assisting with the literature search and to Mr. Oleh Weres of the University Lawrence Berkeley Laboratory for the Poubaix diagram of selenium. Project Officer for this report is Dr. Edwin W. Lee, U.S. Bureau of Reclamation, 2800 Cottage Way, Sacramento, California 95825-1898



APPENDIX A
STATEMENT OF WORK

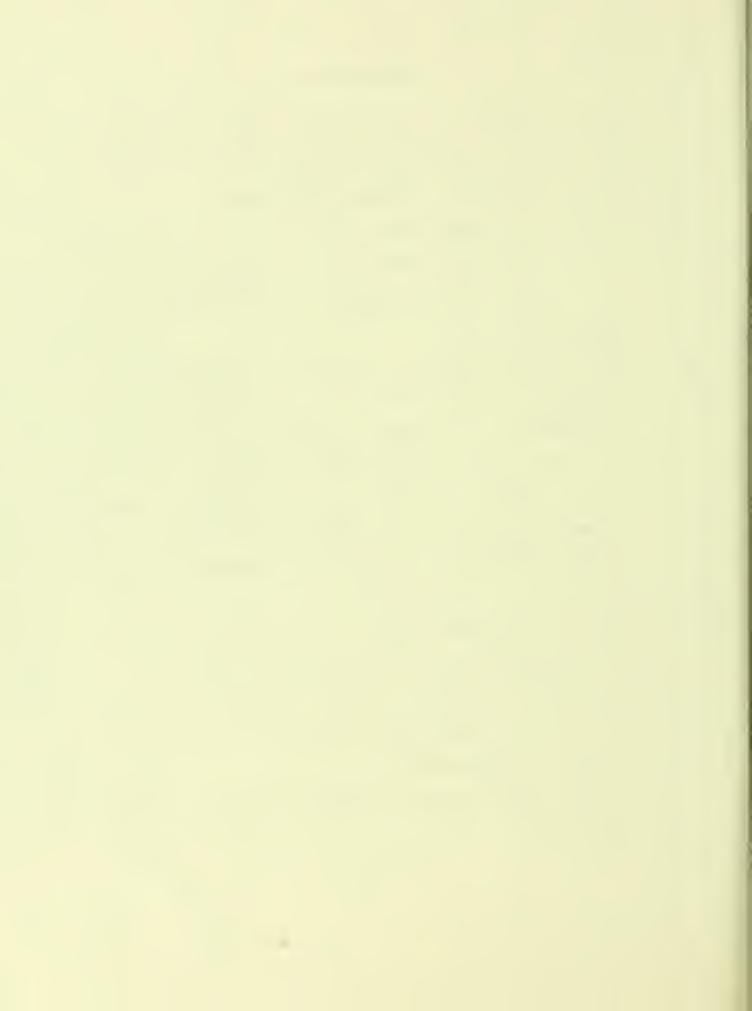


APPENDIX A

STATEMENT OF WORK

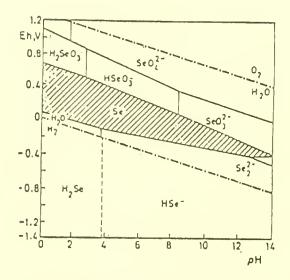
Contractor will make a desk-top study and prepare a technical report on the potential for the treatment of agricultural drainwater by microalgae, covering the following, although not exclusive, aspects:

- 1. Review literature specifically on microalgal systems in the treatment of wastewater, bringing into focus the current state-of-the-art highrate algal ponds and information on current research and operating systems.
- 2. Based on this review, present an analysis of the possible use of microalgae in high-rate pond systems to treat agricultural drainage water, particularly the removal of toxic substances as selenium and the recovery of biomass in energy-efficient systems. Analysis should include integration of high-rate ponds into cost-effective systems as part of the total San Joaquin Valley Drainage Program. A preliminary estimate of the cost to treat drainage water (\$/AF) should be provided.
- 3. Following this analysis, prepare an outline proposal to investigate the use of microalgal high-rate pond systems for agricultural drainage water treatment. The proposal should outline a pilot plant and a preliminary study program directed to investigate the feasibility of these systems.
- 4. Prepare a report to be submitted within 60 days of contract work start.



APPENDIX B
POURBAIX DIAGRAM FOR SELENIUM





Pourbaix diagram for selenium



APPENDIX C

REFERENCES



APPENDIX C

REFERENCES

Abbott, W.E. (1948) "Oxygen Production in Water by Photosynthesis" Sewage Works Journal 20, 538.

Anon, (1985) U.S. Bureau of Reclamation, "Water Quality Analyses West Side - San Joaquin Valley During 1984".

Arndt, E.W. (1971), "Thin Wall Sand Filter for Separation of Algal Suspensions", Bohna Engr and Res., Inc., 240 2nd Street, San Francisco, In-House Report.

Arthur, James F., Randall L. Brown, Bruce A. Butterfield and Joel C. Goldman (1969), "Algal Nutrient Responses in Agricultural Waste Water" in Collected Papers Regarding Nitrates in Agricultural Waste Water Water Pollution Control Research Series 13030 ELY, U.S. Department of the Interior, Federal Water Quality Aministration.

Beck, L. (1985), Personal Communication.

Benemann, J.R., B.L. Koopman, J.C. Weissman, D.M. Eisenberg and W.J. Oswald (1977) "Cultivation on Sewage of Microalgae Harvestable by Microstrainers", ERDA Contract No. W.74-05-Eng-48. Sanitary Engineering Research Laboratory, University of California, Berkeley.

Brown, R.L. (1971) "Bioengineering Aspects of Agricultural Drainage" California State Department of Water Resources Publication No. 174-10.

Brown, R.L. (1975) "The Occurrence and Removal of Nitrogen in Subsurface Agricultural Drainage from San Joaquin Valley, California Water Research 9, 529.

Burlew, J.S., ed., (1953), Algal Culture: From Laboratory to Pilot Plant, Carnegie Institution of Washington, Publication No. 600.

Buttino, W.R., C.H. Banks, K.J. Irgulik, P. Micks, A.E. Wheeler and R.A. Zingard (1984) "Selenium Containing Amino Acids and Proteins in Marine Algae" Phytochemistry 23 11-2445-2452

Butterfield, Bruce A. and James R. Jones (1969) "Harvesting of Algae Grown in Agricultural Waste Water" in <u>Collected Papers Regarding</u>
Nitrates in Agricultural Wastewater. Water Pollution Control Research Series 13030 ELY, U.S. Department of the Interior, Federal Water Quality Administration.

Caldwell, D.H. (1946) "Sewage Oxidation Ponds - Performance, Operation and Design". Sewage Works Journal 18, 433.

Chen, P. (1985) "Thermochemical Processing of Microalgae to Improve Methane Yields". SEERHL Report 85-7, University of California, Berkeley.

- Dodd, J.C. (1972) "Harvesting of Algae with a Paper Precoated Belt-type Filter", Davis, University of California, Doctoral Dissertation.
- Dodd, J.C. (1980) "Harvesting Algae Grown on Pig Wastes in Singapore". Paper presented at a workshop on High-rate Algae ponds held in Singapore 27-29 February, 1980. Text included on microfiche in IDRC-154e, International Development Research Centre, Canada.
- Eisenberg, D.M., J.R. Benemann, R.P. Goebel and T.T. Tiburzi (1979) Proceedings of the First International Symposium of Anaerobic Digestion, University Colleges, Cardiff, Wales, 123-135.
- Farnham, H. (1980) Personal Communication.
- Gennity, J.M., N.R. Bottino, R.H. Zingaro, A.E. Wheeler and K.J. Irgolik (1984) "The Binding of Selenium to the Lipids of Two Unicellular Marine Algae", Biochem. Biophys. Res. Commun. 118, 1, 176-182.
- Giesecks, F.E. and P.J. A. Zeller (1939) "Treatment of Settled Sewage in Lakes", Bulletin No. 47, Texas A. & M. College, 10 1 20.
- Goldman, J.C., L.A. Beck and W.J. Oswald (1969) "Nitrate Removal from Agricultural Tile Drainage by Photosynthetic Systems", <u>Proceedings Second Annual Symposium Sanitary Engineering Research</u>, <u>Development and Design</u>, Cornell University.
- Goldman, Joel C., James F. Arthur, William J. Oswald and Louis A. Beck (1969) "Combined Nutrient Removal and Transport System for Tile Drainage from the San Joaquin Valley" in Collected Papers Regarding Nitrates in Agricultural Wastewater, Water Pollution Control Research Series 13030 ELY, U.S. Department of the Interior, Federal Water Quality Administration.
- Goldman, J.C., W.J. Oswald and D. Jenkins (1974) "The Kinetics of Inorganic Carbon Limited Algal Growth", <u>Journal Water Pollution Control Federation 46</u>, 3 554-574.
- Golueke, C.G., W.J. Oswald and H.B. Gotaas (1957) Methane Fermentation of Algae, Applied Microbiology 5, 47-55.
- Golueke, C.G. and W.J. Oswald (1965) "Harvesting and Processing Sewage-grown Planktonic Algae" Journal Water Pollution Control Federation, 37, (4), 471-498.
- Golueke, C.G. and W.J. Oswald (1968) "Large-scale Production of Algae", Single-Cell Protein, Ed. R.I. Mateles and S.F. Tannenbaum, the M.I.T. Press, Cambridge, Massachusetts.
- Grobbelaar, J.U., C.J. Soeder and E. Stengel (1981) "Modeling Algae Productivity and Oxygen Production in Large Outdoor Cultures", Jul-Spee 282 ISSN 0343-7639 Kernfor Schung Sanlage Julich GMBH Institut fur Biotechnologie.
- Hall, T.W., W.J. Oswald, J.M. Moore and P.H. Chen (1981) "Recovery of Methane from Facultative Sewage Ponds, Final Report", University of California Appropriate Technology Program, Pg. 80, 89, 4000. Sanitary Engineering and Env. Health Research Lab., University of California SEEHRL 81-12-1:1-30.

Herman, E.R. and E.F. Gloyna (1958) "Waste Stabilization Ponds", Sewage and Industrial Wastes 30, 11, 646-693.

Hills, C. (1980) "Spirulina as an Aid in Cell Respiration". Proceedings International Symposium in Biogas Microalgae and Livestock Waste" Ed. Chung Po, PhD, Council for Agricultural Planning and Development, R.O.C., Taipei, Taiwan.

Hintz, H.F., H. Heatman, Jr., W.C. Weir, D.T. Torell and J.H. Meyer, (1966) "Nutritive Value of Algae Grown on Sewage". Journal of Animal Science, 25(3), 675-681.

Jones-Witthuhn, R.L., A.S. Brooks and M.E. Boraas (1983) "The Importance of Sulfur Concentration in Defining the Toxicity of Selenium to the Alga Chlamydomonas reinhardii", Proceedings of 26th Conference on Great Lakes Research, State University of New York at Oswego.

Knight, A.W. and C. Foe (1985) "Selenium Bioaccumulation, Regulation and Toxicity in the Green Alga <u>Selenastrum capricornuta</u> and Dietary Toxicity of the Contaminate Alga to <u>Daphnia magna</u>". Internal Report to the U.S. Bureau of Reclamation.

Kormanik, R.A. and J.B. Cravens (1978) "Remove Algae Through Microscreening", Water and Wastes Engineering, 11.

Krofta Engineering Corporation, Lenox, Massachusetts.

Kumar, H.D. and G. Prakash (1971) "Toxicity of Selenium to the Blue-Green Algae Anacystis nidulans and Anabaena variabilis". Annals of Botany 35, 697-705.

Laskin et al, (1977) "Handbook of Microbiology" CRC Press.

Laws, E.A., K.L. Terry, J. Wickman and M.S. Chalup (1983), "Preliminary Results from a Simple Algal Production System Designed to Utilize the Flashing Light Effect", Biotechnol. Bioengr., Vol. 25, pg 2319.

Laws, E.A. (1984) "Research and Development of Shallow Algal Mass Culture Systems for Production of Oils" SERI/STR 231-249, Solar Energy Research Institute, Golden Colorado.

Lindstrom, K. (1983) "Selenium as a Growth Factor for Plankton Algae in Laboratory Experiments and in Some Swedish Lakes", Hydrobiologia 101, 1 and 2, pp. 35-48.

Ludwig, H.F. and W.J. Oswald (1952) "Role of Algae in Sewage Oxidation Ponds", Scientific Monthly: 74, 3-6.

McGarry, M.G. (1970) "Algal Flocculation with Aluminum Sulfate and Polyelectrolytes", <u>Journal Water Pollution Control Federation</u>, 42-5, 191-201.

McGarry, M.G., C.D. Lin and J.L. Merto (1973), "Photosynthetic Yields and By-product Recovery from Sewage Oxidation Ponds". In Jenkins, S.H., ed., Advances in Water Pollution Research. U.K. 521-535.

McKeown, Brian (1985) "Removal of Nutrients and Trace Elements (particularly selenium) from Agricultural Subsurface Drainage Utilizing Facultative Ponds". Report for Applied Algology and Culture Pond Design BEHS 259, School of Public Health, University of California, Berkeley.

Meron, A., M. Zabat and W.J. Oswald (1970), "Designing Waste Ponds to Meet Water Quality Criteria". Proceedings, Second International Sumposium for Waste Treatment Lagoons, University of Kansas, Manhattan, Kansas.

Moon, A. (1970). Doctoral Dissertation, University of California, Berkeley.

Miller, S.F. and C.R. Wilke (1972), "Utilization of Algae for Water Purification and Protein Production & Photoactive Separation", AEL W-7505-Eng 48, University of California, LBL 289, pp 43-57.

Nurdogan, Y. (1985) "Removal of Autoflocculated Algae in Tube Settlers". University of California SEEHRL Lab News, Quarterly 32 1, University of California, Berkeley.

Oswald, W.J. and H.B. Gotaas (1957), "Photosynthesis in Sewage Treatment". Transactions, American Society of Civil Engineers, 122, 73-105

Oswald, W.J., C.G. Golueke and H.K. Gee (1957), "Waste Water Reclamation Through Production of Algae", University of California Water Resources Center, Contribution No. 22.

Oswald, W.J., (1960), "Light Conversion Efficiency of Algae Grown in Sewage", Journal, Sanitary Engineering Division, ASCE, SA 4 2558, 7.

Oswald, W.J. and C.G. Golueke, (1960) "Biological Transformation of Solar Energy", Advances in Applied Microbiology II, Academic Press.

Oswald, W.J., R.A. Tsugita, R.C. Cooper and C.G. Golueke (1970) "Anaerobic Aerobic Ponds for Beet Sugar Waste Treatment", WPD 93-03 WPD, 93-04 Final Report Beet Sugar Development Foundation.

Oswald, W.J. (1974) "Evaluation of Performance of the Fairfield, Calif. Waste Disposal Ponds", Report by Jenks and Adamson, Palo Alto, California.

Oswald, W.J. and Roy Ramani (1974) "Evaluation of a new Method for Algae Separation", Final Report submitted to Regents of the University of California.

Oswald, W.J. (1975) "Studies of Pond Performance and Pilot Algae Separation at Napa Sanitation District", Report to Napa Sanitation District.

Oswald, W.J. (1977) "Method of Waste Treatment and Algae Recovery", Patent No. 4,005,546, University of California, Berkeley

- Oswald, W.J., E.W. Lee, B. Adam and K.H. Yao (1978) "New Wastewater Treatment Method Yields a Harvest of Saleable Algae", World Health Organization Chronicle, 32: 348-350.
- Oswald, W.J., (1978) "Pilot Plant High-Rate Study . . ." Final Report to World Health Organization and Laguna Lake Development Administration, Philippines.
- Oswald, W.J. (1980) "Algal Production Problems, Achievements and Potential", in Algal Biomass, Elsevier / North Holland, Biomed Press.
- Oswald, W.J. and D.M. Eisenberg (1980) "Biomass Generation Systems as an Energy Resource", Proceedings Bioenergy 80 Conference, Atlanta Bioenergy Council, Washington, D.C.
- Oswald, W.J. and L.E. Swanson (1981) "Biomass Fueled Anaerobic Digestion Systems". Proceedings of NATO Conference on Energy Production and Conservation from Wastes", Borgazici University, Istanbul Turkey, ed., Professor Criton Curi.
- Oswald, W.J. (1985) "Large-scale Algal Culture Systems: Engineering Aspects", in Micro-Algal Biotechnology, Ed., Michael Borowitzka and Lesley Borowitzka, Cambridge University Press. In Press.
- Pappenfuss, G.F. and P.C. Silva (1953) "A Systematic Study of the Algae of Sewage Oxidation Ponds". SWPCB7, State Water Pollution Control Board, Sacramento, California.
- Parker, D.S. (1978) "Performance of Alternative Algae Removal Systems", University of Texas Water Resources Center Symposium No. 9, Ponds as a Wastewater Treatment Alternative, University of Texas, Austin.
- Pavoni, J.L., M.W. Tenney and W.F. Exkelberger, Jr. (1974) "The Relationship of Algal Extracellular Polymers to Biological Flocculation", Proceedings 26th Industrial Waste Conference, Purdue University, Lafayette, Indiana.
- Presser, T.S. and Ivan Barnes (1984) "Selenium Concentrations in Waters Tributary to and in the Vicinity of the Kesterson National Wildlife Refuge, Fresno and Merced Counties, California". Water Resources Investigations Report 84-4112, U.S. Geological Survey, Menlo Park, California.
- P.R.C. Inc. (1983) "Los Banos Demonstration Desalting Facility Final Engineering Report: Physical/Chemical Pretreatment and Reverse Osmosis Desalting Systems", Prepared for State of California Department of Water Resources P.R.C. Eng. 972 Town & Coutry Road, P.O. Box 5367, Orange, California 92667.
- Provasoli, L. and A.F. Carlucci (1974) In Algal Physiology and Biochemistry, Stewart W.D.P., ed. Blackwell Scientific, Oxford and University of California press, Berkeley. pp 741-787.

- Ramani, R. (1974) "Studies of Modesto Sewage Treatment Plant", Doctoral Dissertation, University of California, Berkeley.
- Ramani, R. and W.J. Oswald (1975) "Studies on Pond Performance and Pilot Algae Separation at Napa Sanitation District". CSO International to Napa Sanitation District, 950 Imola Avenue, West Napa, California 94558.
- Saiki, Michael K. (1984) "Selenium Concentrations in Aquatic Food Chain Organisms and Fish Exposed to Agricultural Tile Drainage Water". Proceedings University of California, Berkeley, workshop on Selenium and Agricultural Drainage, Water Resources Library, University of California, Berkeley.
- Saliternick, C. and E. Hiller (1974) "Separation of Algae from Oxidation Ponds by Flotation Produced by Nozzles which Produce Micro-Bubbles", Report, Hebrew University, Jerusalem.
- Sekoulov, Ivan D. (1979) "A pH Controlled Activated Algae System for the Advanced Wastewater Treatment", Progress in Water Technology 11 4/5, pp 353-359, Pergammon Press, LTD.
- Setlik, I. Ed., (1970), Prediction and Measurement of Photosynthetic Productivity Proceedings of the 1969 IBP/PP Technical Meeting, Trebon, Czechoslovakia.
- Shelef, G. R. Moraine, A. Maydan and E. Sandbank (1976), "Combined Algae Production Wastewater Treatment and Reclamation Systems", in H.G. Schegel and J. Barnea (Edms). Microbial. Energy Conversion, Goltze, Goettingen, pp 399-414.
- Shelef, G., A. Sukenik and M. Green (1984) "Microalgae Harvesting and Processing a Literature Review". SERI/STR, 231-239, Solar Energy Research Institute, Golden, Colorado.
- Shelef, G., W.J. Oswald and P.H. McGauhey (1970) "Algal Reactor for Life Support Systems", Journal of Sanitary Division ASCE 96 (SAI) 91-110.
- Shelef, G. (1972) "Assaying Algal Growth with Respect to Nitrate Concentration in a Continuous Flow Turbidostat". In Proceedings, 5th International Water Pollution Research Conference, Pergammon Press, London.
- Shelef, G., E. Sandbank and A.M. Wachs (1973) "Experiments in Integrated Photosynthetic Wastewater Treatment and Algae Production Systems". Proceedings, Conference on Environmental Quality, Haifa, Israel.
- Shelef et al (1980) "Algal Biomass", Elsevier/North Holland Biomedical Press.
- Shifrin, M.S. (1980) "Phytoplankton Lipids: Environmental Influences on Production and Possible Commercial Applications". Doctoral Dissertation, Massachusetts Institute of Technology, Boston, Mass.
- Shrift, A. (1953) "Sulfur Selenium Antagonism I and II, Antimetabolite Action of Selenate on the Growth of Chlorella vulgaris", American Journal of Botany 41, 223-230. Botany, 41, 345-352.

- Shrift, Alex (1958) "Biological Activities of Selenium Compounds", The Botanical Review, 550-583.
- Sielick, M. (1972) "The Effect of Selenium on the Ultra Structure and Physiological Properties of the Blue-Green Alga <u>Phormidium luridum</u>, Var. <u>Olivacea</u>". PB-265 066, National Technical Information Service, Springfield, Virginia 22161.
- Soong, P. (1980) "Cultivation of Spirulina in Swine Waste". Proceedings of the International Symposium on Biogas, Microalgae and Livestock Waste, Taipei, Taiwan, R.O.C.
- Steel, E.W. and E.F. Gloyna (1954) "Oxidation Ponds: Radioactivity Uptake and Algae Concentration", Technical Report No. 1, University of Texas, Sanitary Engineering Laboratory, Austin, Texas.
- Stein, Janet R. (1973) Handbook of Phycological Methods, Culture Methods and Growth Measurements, Cambridge University Press.
- Swanson-Oswald Associates, Inc. (Formerly CSO International, Inc.) (1978) City of Hollister Sewage Treatment Plant Design. 3366 McGraw Lane, Lafayette, California 94549.
- Switzer, L. (1982) "Spirulina, the Whole Food Revolution", Bantam Books, Inc., 666 5th Avenue, NY. New York 10103.
- Tornabene, T.G. A. Ben-Amotz, S. Raziuddin and J. Hubbard (1983) "Chemical Profiles of Microalgae with Emphasis on Lipids", Final Report for SERI Subcontract No. XK-2-02149-1 (portions also published in "Screening for Lipid Yielding Microalgae", Final Subcontract Report SERI/STR-231-2207), Solar Energy Research Institute, Golden, Colorado.
- Uziel, M., W.J. Oswald and C.G. Golueke (1975) "Methane Fermentation of Algae" Final Progress Report NSF RANN, SE/GI 39216FR-75-5.
- Van Muylder, J. and M. Pourbaix (1974) "Selenium Atlas of Electrochemical Equilibria in Aqueous Solutions", Ed. M. Pourbaix, National Association of Corrosion Engineers, Houston, Texas.
- Vocke, R.W., K.L. Sears, J.J. O'Toole and R.B. Wildman (1980) "Growth Responses of Selected Freshwater Algae to Trace Elements and Scrubber Ash Slurry Generated by Coal-Fired Power Plants", Water Research 14, 141-150.
- Wachs, A.M., G. Shelef and E. Sandbank (1973) "Algae of Stabilization Ponds, their Separation and Utilization", Final Report, Environmental Engineering Laboratory, Technion Research Development Foundation, Ltd., Haifa.
- Wang, H.K. and J.M. Wood (1984) "Bioaccumulation of Nickel by Algae", Env. Science and Technology $\underline{18}$ 2, $\underline{106}$ - $\underline{109}$.
- Westberg, G.L. (1985) Moorse Laboratories, 1525 Fulton Avenue, Sacramento, California 95875. Algae analyses 33559, 2/26/85 and 33721, 3/12/85.
- Zabat, M. (1970) "Kinetics of Phosphorus Removal by Algae", Doctoral Dissertation, University of California, Berkeley.

